Antrag auf Erteilung eines europäischen Patents / Request for grant of a European patent / Requête en délivrance d'un brevet europ´en

1

Bestätigung einer bereits durch Telekopie (Telefax) eingereichten Anmeldung / Confirmation of an application already filed by facsimile / Confirmation d'une demande déjà déposée par télécopie
Wenn ja, Datum der Übermittlung der Telekopie und Name der Einreichungsbehörde / If yes, facsimile date and name of the authority with which the documents were filed / Si oui, date d'envoi de la télécopie et nom de l'autorité de dépôt

☐ Ja / Yes / Oui

Datum / Date Behörde / Authority / Autorité

Nur für amtlichen Gebrauch / For official use only / Cadre réservé à l'administration

Anmeldenummer / Application No. / N° de le demande	MKEY	1	98302528.3 ✓
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC	2	-1 APR 1998 ✓
Tag des Eingangs beim EPA (Regel 24(4)) / Date of receipt at EPO (Rule 24(4)) / Date de réception à l'OEB (règle 24)4))	RENA	3	14.04.98 ✓
Anmeldetag / Date of filing / Date de dépôt		4	

Tabulatoren-Positionen / Tabulation marks / Arrêts de tabulation

Es wird die Erteilung eines europäischen Patents und gemäß Artikel 94 die Prüfung der Anmeldung beantragt / Grant of a European patent, and examination of the application under Article 94, are hereby requested / Il est demandé la délivrance d'un brevet européen et, conformément à l'article 94, l'examen de la demande — EXAM 4 — 5 ☒ *Prüfungsantrag in einer zugelassenen Nichtamtssprache (siehe Merkblatt II, 5): / Request for examination in an admissible non-EPO language (see Notes II,5): / Requête en examen dans une langue non officielle autorisée (voir notice II, 5):* ✓

Zeichen des Anmelders oder Vertreters (max. 15 Positionen) / Applicant's or representative's reference (maximum 15 spaces) / Référence du demandeur ou du mandataire (max. 15 caractères ou espaces) — AREF — 6 ACC/GH50016 ✓

ANMELDER / APPLICANT / DEMANDEUR
Name / Nom — 7 SmithKline Beecham Corporation

Anschrift / Address / Adresse — 8 One Franklin Plaza ✓
Philadelphia
PA 19103
United States of America

APPR 01 # 12011214141d
DEST

Zustellanschrift / Address for correspondence / Adresse pour la correspondance — 9
PADR

Staat des Wohnsitzes oder Sitzes / State of residence or of principal place of business / Etat du domicile ou du siège — 10 US

Staatsangehörigkeit / Nationality / Nationalité — 11 US

Telefon / Telephone / Téléphone — 12

Telex / Télex | **Telefax / Fax / Téléfax** — 13

Weitere(r) Anmelder auf Zusatzblatt / Additional applicant(s) on additional sheet / Autre(s) demandeur(s) sur feuille additionnelle — 14 ☐

VERTRETER / REPRESENTATIVE / MANDATAIRE:
Name / Nom — 15 CONNELL, Anthony Christopher CRUMP
(Nur einen Vertreter angeben, der in das europäische Patentregister eingetragen und an den zugestellt wird / Name only one representative, who is to be listed in the Register of European Patents and to whom notification is to be made / N'indiquer qu'un seul mandataire, qui sera inscrit au Registre européen des brevets et auquel signification sera faite)

See letter 02/06/98 ✓

FREP 01 216991411 3 # #

Geschäftsanschrift / Address of place of business / Adresse professionnelle — 16 SmithKline Beecham
Corporate Intellectual Property
Two New Horizons Court
Brentford
Middlesex TW8 9EP
United Kingdom

Telefon / Telephone / Téléphone — 17 +44 1279 64 4395

Telex / Télex | **Telefax / Fax / Téléfax** — 18 +44 181 975 6294

Weitere(r) Vertreter auf Zusatzblatt / Additional representative(s) on additional sheet / Autre(s) mandataire(s) sur feuille additionnelle — 19 ☐

EPA/EPO/OEB Form 1001.1 07.97 — TRAN — FILL — EN

ACC/GH50016
Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

Vollmacht / Authorisation / Pouvoir:

ist beigefügt / is enclosed / ci-joint

ist registriert unter Nummer / has been registered under No. / a été enregistré sous le n° GENA

ERFINDER / INVENTOR / INVENTEUR: INVT 20 # #

Anmelder ist (sind) alleinige(r) Erfinder / The applicant(s) is (are) the sole inventor(s) / Le(s) demandeur(s) est (sont) le (les) seul(s) inventeur(s)

Erfindernennung auf gesondertem Schriftstück / Designation of inventor attached / Voir la désignation de l'inventeur ci-jointe

BEZEICHNUNG DER ERFINDUNG / TITLE OF INVENTION / TITRE DE L'INVENTION:

TIDE | TIEN | TIFR

PRIORITÄTSERKLÄRUNG / DECLARATION OF PRIORITY / DECLARATION DE PRIORITE PRIO

01 # . . # . . . . . #

02 # . . # . . . . . #

03 # . . # . . . . . #

04 # . . # . . . . . #

Weitere Prioritätserklärung(en) auf Zusatzblatt / Additional declaration(s) of priority on additional sheet / Autre(s) déclaration(s) de priorité sur feuille additionnelle

BIOLOGISCHES MATERIAL

Die Erfindung betrifft biologisches Material oder seine Verwendung, das nach Regal 28 hinterlegt worden ist.

BIOLOGICAL MATERIAL

The invention relates to and/or uses biological material deposited under Rule 28.

BIOM 1 # | | | | | | #

Die Angaben nach Regel 28(1) c) sind in den technischen Anmeldungsunterlagen enthalten auf / The particulars referred to in Rule 28(1) (c) are given in the technical documents in the application on / Les indications visées à la règle 28(1) c) figurent dans les pièces techniques de la demande à la / aux

werden später mitgeteilt / will be submitted later / seront communiquées ultérieurement

Die Empfangsbescheinigung(en) der Hinterlegungsstelle ist (sind) beigefügt / The receipt(s) of deposit issued by the depositary institution is (are) enclosed / Le(s) récépissé(s) de dépôt délivré(s) par l'autorité de dépôt est (sont) ci-joint(s)

wird (werden) nachgereicht / will be filed later / sera (seront) produit(s) ultérieurement

Verzicht auf die Verpflichtung des Antragstellers nach Regel 28(3) auf gesondertem Schriftstück / Waiver of the right to an undertaking from the requester pursuant to Rule 28(3) attached / Renonciation, sur document distinct, à l'engagement du requérant au titre de la règle 28(3)

20 A. FIEDLER 20 -04- 1998

21 C5630.07 371270.0 Nummer / Number / Numéro

P. BEARZATTO CRISTOFORI 18 JUNI 1998

22

23 x

24 A TNF HOMOLOGUE, TL5

25

Staat / State / Etat	Anmeldetag / Filing date / Date de dépôt	Aktenzeichen / Application No. / N° de la demande
1 US	2 April 1997 02.04.97	60/041,797
2 US	3 December 1997 03.12.97	08/984,396
3		
4		

26 MATIERE BIOLOGIQUE

L'invention concerne et/ou utilise la matière biologique, déposée conformément à la règle 28.

27

Seite(n) / page(s)	Zeile(n) / line(s) / ligne(s)

27a

28

28a

29

ACC/GH50016

Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

Falls das biologische Material nicht vom Anmelder, sondern von einem Dritten hinterlegt wurde: / Where the biological material has been deposited by a person other than the applicant: / Lorsque la matière biologique a été déposée par une personne autre que le demandeur:

Ermächtigung nach Regel 28(1)d) / Authorisation under Rule 28(1)(d) / Autorisation en vertu de la règle 28(1)d)

ist beigefügt / is enclosed / ci-jointe 30a ☐

wird nachgereicht / will be filed later / sera produite ultérieurement 30b ☐

30 Name und Anschrift des Hinterlegers / Name and address of depositor / Nom et adresse du déposant :

NUCLEOTID-UND AMINOSÄURESEQUENZEN / NUCLEOTIDE AND AMINO ACID SEQUENCES / SEQUENCES DE NUCLEOTIDES ET D'ACIDES AMINES SEQL(1)

31

Die Beschreibung enthält ein Sequenzprotokoll nach Regel 27a(1) / The description contains a sequence listing in accordance with Rule 27a(1) / La description contient une liste de séquences selon la règle 27bis(1) ☒

Der vorgeschriebene maschinenlesbare Datenträger ist beigefügt / The prescribed machine readable data carrier is enclosed / Le support de données prescrit déchiffrable par machine est annexé ☒

Es wird hiermit erklärt, daß die auf dem Datenträger gespeicherte Information mit dem schriftlichen Sequenzprotokoll übereinstimmt (Regel 27a(2)) / It is hereby stated that the information recorded on the data carrier is identical to the written sequence listing (Rule 27a(2)) /Il est déclaré par la présente que l'information figurant sur le support de données est identique à celle que contient la liste de séquences écrite (règle 27bis (2)) ☒

32

BENENNUNG DER VERTRAGSSTAATEN UND ERKLÄRUNGEN HIERZU

1. Hiermit werden sämtliche Vertragsstaaten des EPÜ bennant, die bei Einreichung dieser Anmeldung dem EPÜ angehören.

2. Der Anmelder beabsichtigt derzeit, Benennungsgebühren für die nachfolgende angekreuzten Vertragsstaaten zu entrichten:

DESIGNATION OF THE CONTRACTING STATES AND ASSOCIATED DECLARATIONS

1. All states which are Contracting States to the EPC at the filing of this application are hereby designated.

2. The applicant currently intends to pay designation fees for the States marked below with a cross:

DEST

☐	AT	Österreich / Austria / Autriche
☒	BE	Belgien / Belgium / Belgique
☒	CH/LI	Schweiz und Liechtenstein / Switzerland and Liechtenstein / Suisse et Liechtenstein
☒	DE	Deutschland / Germany / Allemagne
☒	DK	Dänemark / Denmark / Danemark
☐	ES	Spanien / Spain / Espagne
☐	FI	Finnland / Finland / Finlande
☒	FR	Frankreich / France / France
☐	—	_____
☐	—	_____

(Platz für Vertragsstaaten, für die das EPÜ nach Drucklegung dieses Formblatts in Kraft tritt / Space for Contracting States for which the EPC enters into force after this form has been printed / Prévu pour des Etats contractants à l'égard desquels la CBE entrera en vigueur après l'impression du présent formulaire)

Es wird beantragt, für die unter Nr. 2 nicht angekreuzten Vertragsstaaten von der Zustellung von Mitteilungen nach Regel 85a (1) und Regel 69 (1) abzusehen.
Ist ein **automatsicher Abbuchungsauftrag** erteilt worden (Feld 43), so wird beantragt, bei Ablauf der Grundfrist nach Artikel 79 (2) EPÜ Benennungsgebühren nur für die unter Nr.2 angekreuzten Vertragsstaaten abzubuchen.

It is requested that no communications under Rule 85a (1) and Rule 69 (1) be notified concerning the Contracting States not marked with a cross under Nr.2.
If an **automatic debit order** has been given (section 43), it is requested that, when the basic period specified in Art. 79(2) expires, designation fees be debited only for the Contracting States marked with a cross under No.2.

DESIGNATION D'ETATS CONTRACTANTS ET DECLARATIONS A CE PROPOS

☒ 1. Sont désignés tous les Etats qui sont des Etats contractants de la CBE à la date du dépôt de la présente demande .

2. Le demandeur se propose actuellement de payer des taxes de désignation pour les Etats cochés ci-dessous:

☒	GB	Vereinigtes Königreich / United Kingdom / Royaume-Uni
☐	GR	Griechenland / Greece / Grèce
☐	IE	Irland / Ireland / Irlande
☒	IT	Italien / Italy / Italie
☐	LU	Luxemburg / Luxembourg / Luxembourg
☐	MC	Monaco / Monaco / Monaco
☒	NL	Niederlande / Netherlands / Pays-Bas
☐	PT	Portugal / Portugal / Portugal
☐	SE	Schweden / Sweden / Suède
☐	—	_____
☐	—	_____

(Platz für Vertragsstaaten, für die das EPÜ nach Drucklegung dieses Formblatts in Kraft tritt / Space for Contracting States for which the EPC enters into force after this form has been printed / Prévu pour des Etats contractants à l'égard desquels la CBE entrera en vigueur après l'impression du présent formulaire)

☒ Prière de ne pas procéder à la signification des notifications prévues par les règles 85bis (1) et 89 (1) pour les Etats contractants n'ayant pas été cochés au no 2.
Si un **ordre de prélèvement automatique** a été donné (rubrique 43), prière de ne prélever à l'expiration des délais de base tels que définis à l'article 79(2) que les taxes de désignation pour les Etats contractants cochés au no.2.

ACC/GH50016

Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

Verschiedene Anmelder für verschiedene Vertragsstaaten / Different applicants for different Contracting States / Différents demandeurs pour différents Etats contractants

APPR 02 # | | | | | | | # | | | | | | | | | |

33 Name(n) des (der) Anmelder(s) und benannte Vertragsstaaten / Name(s) of applicant(s) and designated Contracting States / Nom(s) du (des) demandeur(s) et des Etats contractants désignés

ERSTRECKUNG DES EUROPÄISCHEN PATENTS

Diese Anmeldung gilt als Antrag, die europäische Patentanmeldung und das darauf erteilte europäische Patent auf alle Nicht-Vertragsstaaten des EPÜ zu erstrecken, mit denen am Tag ihrer Einreichung „Erstreckungsabkommen" bestehen (Derzeit: Albanien, Litauen, Lettland, Rumänien, Slowenien). Die Erstreckung wird jedoch nur wirksam, wenn die vorgeschriebene Erstreckungsgebühr entrichtet wird.

EXTENSION OF THE EUROPEAN PATENT

This application is deemed to be a request to extend the European patent application and the European patent granted in respect of it to all non-Contracting States to the EPC with which "extension agreements" exist on the date on which the application is filed (Present situation: Albania, Lithuania, Latvia, Romania, Slovenia). However, the extension only takes effect if the prescribed extension fee is paid

EXPT

34

EXTENSION DES EFFETS DU BREVET EUROPEEN

La présente demande est réputée constituer une requête en extension des effets de la demande de brevet européen et du brevet européen délivré sur la base de cette demande à tous les Etats non partie s à la CBE avec lesquels il existe un «accord d'extension» à la date du dépôt de la demande (Situation actuelle : Albanie, Lituanie, Lettonie, Roumanie, Slovénie). Toutefois l'extension ne produit ses effets que s'il est acquitté la taxe d'extension prescrite.

Der Anmelder beabsichtigt derzeit, die Erstreckungsgebühr für die nachfolgend angekreuzten Staaten zu entrichten: / The applicant currently intends to pay the extension fee for the States marked below with a cross: / Le demandeur se propose actuellement d'acquitter la taxe d'extension pour les Etats dont le nom est coché ci-après:

Albanien / Albania / Albanie	AL	☐
Litauen / Lithuania / Lituanie	LT	☐
Lettland / Latvia / Lettonie	LV	☐
Rumänien / Romania / Roumanie	RO	☐
Slowenien / Slovenia / Slovénie	SI	☐
		☐
		☐
		☐

(Platz für Staaten, mit denen nach Drucklegung dieses Formblatts „Erstreckungsabkommen" in Kraft treten) / (Space for States with which "extension agreements" enter into force after this form has been printed) / (Prévu pour des Etats à l'égard desquels des «accords d'extension» entreront en vigueur après l'impression du présent formulaire)

Die Anmeldung ist eine Teilanmeldung / The application is a divisional application / La présente demande constitue une demande divisionnaire

DFIL 9 | | | | | # |

PANR | | | | | | | # |

35 ☐ Nummer der früheren Anmeldung / No. of earlier application / Numéro de la demande initiale

Es handelt sich um eine Anmeldung nach Art. 61(1)b) / The application is an Art. 61(1)(b) application / Le présente demande constitue une demande selon l'article 61(1)b)

DFIL 9 | | | | | # |

EANR | | | | | | | # |

36 ☐ Nummer der früheren Anmeldung / No. of earlier application / Numéro de la demande initiale

Patentansprüche / Claims / Revendications CLMS

37 19 Zahl der Patentansprüche / Number of claims / Nombre de revendications ✓

Zur Veröffentlichung mit der Zusammenfassung wird vorgeschlagen Abbildung Nr. / With the abstract it is proposed to publish figure No. / Il est proposé de publier avec l'abrégé la figure n° DRAW (2)

39 ☐ Nummer / Number / Numéro

Zusätzliche Abschrift(en) der im europäischen Recherchenbericht angeführten Schriftstücke wird (werden) beantragt / Additional copy(ies) of the documents cited in the European search report is (are) requested / Prière de fournir une (des) copie(s) supplémentaire(s) des documents cités dans le rapport de recherche européenne

ASOC

40 One

Anzahl der zusätzlichen Sätze von Abschriften
Number of additional sets of copies
Nombre de jeux supplémentaires de copies

✓

Es wird die Rückerstattung der Recherchengebühr gemäß Art. 10 GebO beantragt / Refund of the search fee is requested pursuant to Article 10 of the Rules relating to Fees / Le remboursement de la taxe de recherche est demandé en vertu de l'article 10 du règlement relatif aux taxes

41

Eine Kopie des Recherchenberichts ist beigefügt / A copy of the search report is attached / Une copie du rapport de recherche est jointe

42

AUTOMATISCHER ABBUCHUNGSAUFTRAG *(nur möglich für Inhaber von beim EPA geführten laufenden Konten)*
AUTOMATIC DEBIT ORDER *(for EPO deposit account holders only)*
ORDRE DE PRELEVEMENT AUTOMATIQUE *(uniquement possible pour les titulaires de comptes courants ouverts auprès de l'OEB)*

Das Europäische Patentamt wird hiermit beauftragt, fällig werdende Gebühren und Auslagen nach Maßgabe der Vorschriften über das automatische Abbuchungsverfahren vom nebenstehenden laufenden Konto abzubuchen / The European Patent Office is hereby authorised, under the Arrangements for the automatic debiting procedure, to debit from the deposit account opposite any fees and costs falling due / Par la présente, il est demandé à l'Office européen des brevets de prélever du compte courant ci-contre les taxes et frais venant à échéance, conformément à la réglementation relative au prélèvement automatique

DECA

FÜR AUTOMATISCHEN ABBUCHUNGSAUFTRAG:
FOR AUTOMATIC DEBIT ORDER:
POUR L'ORDRE DE PRELEVEMENT AUTOMATIQUE

Nummer des laufenden Kontos / Deposit account number / Numéro du compte courant

Name des Kontoinhabers / Account holder's name / Nom du titulaire du compte

43 28050015

SmithKline Beecham

✓

Eventuelle RÜCKZAHLUNGEN auf das nebenstehende beim EPA geführte laufende Konto / REIMBURSEMENT, if any, to EPO deposit account opposite / REMBOURSEMENTS éventuels à effectuer sur le compte courant ci-contre ouvert auprès de l'OEB

DEPA

Nummer des laufenden Kontos / Deposit account number / Numéro du compte courant

Name des Kontoinhabers / Account holder's name / Nom du titulaire du compte

44 28050015

SmithKline Beecham

✓

Die vorgeschriebene Liste über die diesem Antrag beigefügten Unterlagen ergibt sich aus der vorbereiteten Empfangsbescheinigung (Seite 6 dieses Antrages)

The prescribed list of documents enclosed with this request is shown on the prepared receipt (page 6 of this request)

45 La liste prescrite des documents joints à cette requête figure sur le récépissé préétabli (page 6 de la présente requête)

Unterschrift(en) des (der) Anmelder(s) oder Vertreter(s) / Signature(s) of applicant(s) or representative(s) / Signature(s) du (des) demandeur(s) ou du (des) mandataire(s)

46 Für Angestellte nach Artikel 133(3) Satz 1 mit allgemeiner Vollmacht / For employees under Article 133(3), 1st sentence, having a general authorisation / Pour les employés mentionnés à l'article 133(3), 1ère phrase, munis d'un pouvoir général
Nr. No. / n°:

5630

Ort / Place / Lieu: Harlow, Essex, England

Datum / Date: 1 April 98

A Connell

CONNELL, Anthony Christopher
European Patent Attorney - Agent for the Applicant

✓

Name des (der) Unterzeichneten bitte mit Schreibmaschine wiederholen. Bei juristischen Personen bitte die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft mit Schreibmaschine angeben. / Please type name under signature. In case of legal persons, the position of the signatory within the company should also be typed. / Le ou les noms des signataires doivent être également dactylographiés S'il s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires sera indiquée à la machine à écrire.

ACC/GH50016

Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence au demandeur

Empfangsbescheinigung / Receipt for documents / Récépissé de documents

6

(Liste der diesem Antrag beigefügten Unterlagen) (Checklist of enclosed documents) (Liste des documents annexés à la présente requête)

Es wird hiermit der Empfang der unten bezeichneten Dokumente bescheinigt / Receipt of the documents indicated below is hereby acknowledged / Nous attestons le dépôt des documents désignés ci-dessous

Wird im Falle der Einreichung der europäischen Patentanmeldung bei einer nationalen Behörde diese Empfangsbescheinigung vom Europäischen Patentamt übersandt, so ist sie als Mitteilung gemäß Regel 24(4) anzusehen (siehe Feld RENA). **Nach Erhalt der Mitteilung nach Regel 24(4) sind alle weiteren Unterlagen, die die Anmeldung betreffen, nur noch unmittelbar beim EPA einzureichen.** / If this receipt is issued by the European Patent Office and the European patent application was filed with a national authority it serves as a communication under Rule 24(4) (see Section RENA). **Once the communication under Rule 24(4) has been received, all further documents relating to the application must be sent directly to the European Patent Office.** / Si, en cas de dépôt de la demande de brevet européen auprès d'un service national, l'Office européen des brevets délivre le présent récépissé de documents, ce récépissé est réputé être la notification visée à la règle 24(4). **Dès que la notification visée à la règle 24(4) a été reçue, tous les autres documents relatifs à la demande doivent être adressés directement à l'OEB.**

SmithKline Beecham
Corporate Intellectual Property
Two New Horizons Court
Brentford
Middlesex TW8 9EP
United Kingdom

Nur für amtlichen Gebrauch / For official use only / Cadre réservé à l'administration

Datum / Date

THE PATENT OFFICE
01 APR 1998
LONDON

Unterschrift / Amtsstempel / Signature / Official stamp / Signature / Cachet officiel

Anmeldenummer / Application No. / N° de à demande			98302526.3
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC		-1 APR 1998
Zeichen des Anmelders/Vertreters / Applicant's / Representative's ref. / Référence du demandeur ou du mandataire	AREF		ACC/GH50016
Nur nach Einreichung der Anmeldung bei einer nationalen Behörde: / Only after filing of the application with a national authority: / Seulement après le dépôt de la demande auprès d'un service national:			
Tag des Eingangs beim EPA (Regel 24(4)) / Date of receipt at EPO (Rule 24(4)) / Date de réception à l'OEB (règle 24(4))	RENA		

			Stückzahl / Number of copies / Nombre d'exemplaire	Blattzahl* eines Stücks / Number of sheets* in each copy / Nombre de feuilles* par exemplaire	Gesamtzahl der Abbildungen* / Total number of figures* / Nombre total de figures*
A.	**Anmeldungsunterlagen und Prioritätsbeleg(e) / Application documents and priority document(s) / Pièces de la demande et document(s) de priorité**	47			
1.	Beschreibung / Description		3	29	
2.	Patentansprüche / Claim(s) / Revendication(s)		3	3	
3.	Zeichnung(en) / Drawing(s) / Dessin(s)		-	-	
4.	Zusammenfassung / Abstract / Abrégé — DRAW 1 #		3	1	
5.	Übersetzung der Anmeldungsunterlagen / Translation of the application documents / Traduction des pièces de la demande				
6.	Prioritätsbeleg(e) / Priority document(s) / Document(s) de priorité				
7.	Übersetzung des (der) Priortätsbelegs(belege) / Translation of priority document(s) / Traduction du (des) document(s) de priorité				
B.	**Der Anmeldung in der eingereichten Fassung liegen folgende Unterlagen bei: / This application as filed is accompanied by the items below: / A la présente demande sont annexées les pièces suivantes:**	48			
1.	Einzelvollmacht / Specific authorisation / Pouvoir particulier				
2	Allgemeine Vollmacht / General authorisation / Pouvoir général				
3.	Erfindernennung / Designation of inventor / Désignation de l'inventeur		x		
4	Früherer Recherchenbericht / Earlier search report/ Rapport de recherche antérieure				
5.	Gebührenzahlungsvordruck (EPA Form 1010) / Voucher for the settlement of fees (EPO Form 1010) / Bordereau de règlement de taxes (OEB Form 1010)				
6.	Scheck *(ausgeschlossen bei Einreichung bei den nationalen Behörden)* / Cheque *(not when filing with national authorities)* / Chèque *(pas de chèque en cas de dépôt auprès des services nationaux)*				
7.	Datenträger für Sequenzprotokoll / Data carrier for sequence listing / Support de données pour liste de séquences — SEQL (4)				
8.	Zusatzblatt / Additional sheet / Feuille additionnelle				
9.	Sonstige Unterlagen (bitte hier spezifizieren) / Other (please specify here) / Autres documents (veuillez préciser ici)				
C.	**Kopien dieser Empfangsbescheinigung / Copies of this receipt for documents / Copies du présent récépissé de documents**	49	Two	Anzahl der Kopien / Number of copies/ Nombre de copies	

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98302526.3

A TNF homologue, TL5

This application claims the benefit of U.S. Provisional Application No: 60/041,797, filed April 2, 1997.

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to TNF family, hereinafter referred to as TL5. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

Many biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intracellular response.

For example, tumor necrosis factors (TNF) alpha and beta are cytokines which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-ligands, the "TNF-receptor" superfamily. So far, nine members of the TNF ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized.

Among the ligands there are included are TNF-α, lymphotoxin-α (LT-α, also known as TNF-β), LT-β (found in complex heterotrimer LT-α2-β), FasL, CD40L, CD27L, CD30L, 4-1BBL, OX40L and TRAIL. The superfamily of TNF receptors includes the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-1BB, OX40, low affinity p75 and NGF-receptor (Meager, A., Biologicals, 22:291-295 (1994)).

Many members of the TNF-ligand superfamily are expressed by activated T-cells, implying that they are necessary for T-cell interactions with other cell types which underlie cell ontogeny and functions. (Meager, A., supra).

Considerable insight into the essential functions of several members of the TNF family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand

cause lymphoproliferative disease (Watanabe-Fukunaga, R., et al., Nature 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglubulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R.C. et al., Science 259:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innovation of peripheral structures (Lee, K.F. et al, Cell 69:737 (1992)).

TNF and LT-α are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT-α, acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT-α are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmuine disease, AIDS and graft-host rejection (Beutler, B. and Von Huffel, C., Science 264:667-668 (1994)). Mutations in the p55 Receptor cause increased susceptibility to microbial infection.

Moreover, an about 80 amino acid domain near the C-terminus of TNFR1 (P55) and Fas was reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia et al., Cell 74:845 (1993)).

The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence biological activity, both normally and in disease states. In particular, there is a need to isolate and characterize novel members of the TNF family.

This indicates that the TNF family has an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further members of TNF family which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to TL5 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such TL5 polypeptides and polynucleotides. Such uses include the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with TL5 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate TL5 activity or levels.

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"TL5" refers, among others, generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 or an allelic variant thereof.

"TL5 activity or TL5 polypeptide activity" or "biological activity of the TL5 or TL5 polypeptide" refers to the metabolic or physiologic function of said TL5 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said TL5.

"TL5 gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include,

without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation,

oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press,

New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al., Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al., J Molec Biol* (1990) 215:403).

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5 or 3 terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among

residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Polypeptides of the Invention

In one aspect, the present invention relates to TL5 polypeptides (or TL5 proteins) . The TL5 polypeptides include the polypeptide of SEQ ID NOS:2 and 4; as well as polypeptides comprising the amino acid sequence of SEQ ID NO: 2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within TL5 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and still more preferably at least 95% identity to SEQ ID NO:2. Furthermore, those with at least 97-99% are highly preferred. Preferably TL5 polypeptide exhibit at least one biological activity of TL5.

The TL5 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the TL5 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned TL5 polypeptides. As with TL5 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of TL5 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of TL5 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional

attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate TL5 activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the TL5, including antigenic activity. Among the most preferred fragment is that having the amino acid sequence of SEQ ID NO: 4. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The TL5 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to TL5 polynucleotides. TL5 polynucleotides include isolated polynucleotides which encode the TL5 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, TL5 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 encoding a TL5 polypeptide of SEQ ID NO: 2, and polynucleotides having the particular sequences of SEQ ID NOS:1 and 3. TL5 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the TL5 polypeptide of SEQ ID NO:2, and a polynucleotide comprising a nucleotide sequence that is at least 80% identical to that of SEQ ID NO:1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at

least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under TL5 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such TL5 polynucleotides.

TL5 of the invention is structurally related to other proteins of the TNF family, as shown by the results of sequencing the cDNA encoding human TL5. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 154 to 1008) encoding a polypeptide of 285 amino acids of SEQ ID NO:2. The amino acid sequence of Table 1 (SEQ ID NO:2) has about 34% identity (using BLAST) in 35 amino acid residues with Canis familiaris TNF. Swissprot accession no. P51742/TNFA_CANFA. The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 99% identity (using BLAST) in 376 nucleotide residues with Homo sapiens cDNA clone 593690 3' (Genbank Accession No. AA166695). Furthermore, TL5 (SEQ ID NO:1) is 97% identical to human STS SHGC-36171 over 290 nucleotide base residues (Genbank Accesssion No. G30081). Thus, TL5 polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

Table 1[a]

```
   1  CACGAGAAAA TTCAGGATAA CTCTCCTGAG GGGTGAGCCA AGCCCTGCCA

  51  TGTAGTGCAC GCAGGACATC AACAAACACA GATAACAGGA AATGATCCAT

 101  TCCCTGTGGT CACTTATTCT AAAGGCCCCA ACCTTCAAAG TTCAAGTAGT

 151  GATATGGATG ACTCCACAGA AAGGGAGCAG TCACGCCTTA CTTCTTGCCT

 201  TAAGAAAAGA GAAGAAATGA AACTGAAGGA GTGTGTTTCC ATCCTCCCAC

 251  GGAAGGAAAG CCCCTCTGTC CGATCCTCCA AAGACGGAAA GCTGCTGGCT

 301  GCAACCTTGC TGCTGGCACT GCTGTCTTGC TGCCTCACGG TGGTGTCTTT

 351  CTACCAGGTG GCCGCCCTGC AAGGGGACCT GGCCAGCCTC CGGGCAGAGC

 401  TGCAGGGCCA CCACGCGGAG AAGCTGCCAG CAGGAGCAGG AGCCCCCAAG
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 451  GCCGGCCTGG AGGAAGCTCC AGCTGTCACC GCGGGACTGA AAATCTTTGA

 501  ACCACCAGCT CCAGGAGAAG GCAACTCCAG TCAGAACAGC AGAAATAAGC

 551  GTGCCGTTCA GGGTCCAGAA GAAACAGTCA CTCAAGACTG CTTGCAACTG

 601  ATTGCAGACA GTGAAACACC AACTATACAA AAAGGATCTT ACACATTTGT

 651  TCCATGGCTT CTCAGCTTTA AAAGGGGAAG TGCCCTAGAA GAAAAAGAGA

 701  ATAAAATATT GGTCAAAGAA ACTGGTTACT TTTTTATATA TGGTCAgGTT

 751  TTATATACTG ATAAGACCTA CGCCATGGGA CATCTAATTC AGAGGAAGAA

 801  GGTCCATGTC TTTGGGGATG AATTGAGTCT GGTGACTTTG TTTCGATGTA

 851  TTCAAAATAT GCCTGAAACA CTACCCAATA ATTCCTGCTA TTCAGCTGGC

 901  ATTGCAAAAC TGGAAGAAGG AGATGAACTC CAACTTGCAA TACCAAGAGA

 951  AAATGCACAA ATATCACTGG ATGGAGATGT CACATTTTTT GGTGCATTGA

1001  AACTGCTGTG ACCTACTTAC ACCATGTCTG TAGCTATTTT CCTCCCTTTC

1051  TCTGTACCTC TAAGAAGAAA GAATCTAACT GAAAATACCA AAA
```

[a] A nucleotide sequence of a human TL5 (SEQ ID NO: 1).

Table 2[b]

```
   1  MDDSTEREQS RLTSCLKKRE EMKLKECVSI LPRKESPSVR SSKDGKLLAA

  51  TLLLALLSCC LTVVSFYQVA ALQGDLASLR AELQGHHAEK LPAGAGAPKA

 101  GLEEAPAVTA GLKIFEPPAP GEGNSSQNSR NKRAVQGPEE TVTQDCLQLI

 151  ADSETPTIQK GSYTFVPWLL SFKRGSALEE KENKILVKET GYFFIYGQVL

 201  YTDKTYAMGH LIQRKKVHVF GDELSLVTLF RCIQNMPETL PNNSCYSAGI

 251  AKLEEGDELQ LAIPRENAQI SLDGDVTFFG ALKLL
```

[b] An amino acid sequence of a human TL5 (SEQ ID NO: 2).

One polynucleotide of the present invention encoding TL5 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human Fetal liver spleen, chronic lymphocytic leukemia spleen, ovarian cancer, stomach cancer, smooth muscle cells, neutrophils, PMA stimulated T cells, oxidized LDL stimulated macrophages, dendritic cells, bone marrow cells and cell lines, and CD34+cord blood using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al.*, *Nature*, (1992) *355*:632-634; Adams, M.D., *et al.*, *Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding TL5 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 154 to 1008 of SEQ ID NO:1), or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of TL5 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding TL5 variants comprising the amino acid sequence of TL5 polypeptide of Table 2 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination. Among the preferred polynucleotides of the present invention is contained in Table 3 (SEQ ID NO: 3) encoding the amino acid sequence of Table 4 (SEQ ID NO: 4).

Table 3[c]

```
1  GGGAGAAGGC AACTCCAGTC AGAACAGCAG AAATAAGCGT GCCGTTCAGG
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  51  GTCCAGAAGA AACAGGATCT TACGAGACAT TTGTTCCATG GCTTCTCAGC

 101  TTTAAAAGGG GAAGTGCCCT AGAAGAAAAA GAGAATAAAA TATTGGTCAA

 151  AGAAACTGGT TACTTTTTTA TATATGGTCA GGTTTTATAT ACTGATAAGA

 201  CCTACGCCAT GGGACATCTA ATTCAGAGGA AGAAGGTCCA TGTCTTTGGG

 251  GATGAATTGA GTCTGGTGAC TTTGTTTCGA TGTATTCAAA ATATGCCTGA

 301  AACACTACCC AATAATTCCT GCTATTCAGC TGGCATTGCA AAACTGGAAG

 351  AAGGAGATGA ACTCCAACTT GCAATACCAA GAGAAAATGC ACAAATATCA

 401  CTGGATGGAG ATGTCACATT TTTTGGTGCA TTGAAACTGC TGTGACCTAC

 451  TTACACCATG TCTGTAGCTA TTTTCCTCCC TTTCTCTGTA CCTCTAAGAA

 501  GAAAGAATCT AACTGAAAAT ACCAAAAAAA
```

[c] A partial nucleotide sequence of a human TL5 (SEQ ID NO: 3).

Table 4[d]

```
   1  GEGNSSQNSR NKRAVQGPEE TGSYETFVPW LLSFKRGSAL EEKENKILVK

  51  ETGYFFIYGQ VLYTDKTYAM GHLIQRKKVH VFGDELSLVT LFRCIQNMPE

 101  TLPNNSCYSA GIAKLEEGDE LQLAIPRENA QISLDGDVTF FGALKLL
```

[d] A partial amino acid sequence of a human TL5 (SEQ ID NO: 4).

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more preferably 97-99% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof (including that of SEQ ID NO:3), may

be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding TL5 polypeptide and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than human) that have a high sequence similarity to the TL5 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

In one embodiment, to obtain a polynucleotide encoding TL5 polypeptide, including homologs and orthologs from species other than human, comprises the steps of screening an appropriate library under stingent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof (including that of SEQ ID NO: 3), and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Thus in another aspect, TL5 polynucleotides of the present invention further include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having SEQ ID NO: 1 or a fragment thereof (including that of SEQ ID NO:3). Also included with TL5 polypeptides are polypeptide comprising amino acid sequence encoded by nucleotide sequence obtained by the above hybridization condition. Stringent hybridization conditions are as defined above or, alternatively, conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the TL5 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If TL5 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

TL5 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of TL5 polynucleotides for use as diagnostic reagents. Detection of a mutated form of TL5 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of TL5. Individuals carrying mutations in the TL5 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled TL5 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising TL5 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee *et al.*, *Science*, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory

bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease through detection of mutation in the TL5 gene by the methods described.

In addition, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of TL5 polypeptide or TL5 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an TL5 polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagonostic kit for a disease or suspectability to a disease, particularly chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, which comprises:

(a) a TL5 polynucleotide, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof ;

(b) a nucleotide sequence complementary to that of (a);

(c) a TL5 polypeptide, preferably the polypeptide of SEQ ID NO: 2, or a fragment thereof; or

(d) an antibody to a TL5 polypeptide, preferably to the polypeptide of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes

according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

The gene for TL5 was localized to chromosome 13.

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the TL5 polypeptides. The term "immunospecific" means that the antibodies have substantiall greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the TL5 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against TL5 polypeptides may also be employed to treat chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease,

psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with TL5 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering TL5 polypeptide via a vector directing expression of TL5 polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a TL5 polypeptide wherein the composition comprises a TL5 polypeptide or TL5 gene. The vaccine formulation may further comprise a suitable carrier. Since TL5 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation instonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

The TL5 polypeptide of the present invention may be employed in a screening process for compounds which stimulate (agonists) or inhibit (antagonists, or otherwise called inhibitors) the binding, synthesis or action of the TL5 polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess or identify agonist or antagonists from, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These agonists or antagonists may be natural substrates, ligands, receptors, etc., as the case may be, of the polypeptide of the present invention; or may be structural or functional mimetics of the polypeptide of the present invention. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

TL5 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate TL5 polypeptide on the one hand and which can inhibit the function of TL5 polypeptide on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease. Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease.

In general, such screening procedures may involve using appropriate cells which express the TL5 polypeptide or respond to TL5 polypeptide of the present invention. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells which express the TL5 polypeptide (or cell membrane containing the expressed polypeptide) or respond to TL5 polypeptide are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. The ability of the cells which were contacted with the candidate compounds is compared with the same cells which were not contacted for TL5 activity.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the TL5 polypeptide is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the TL5 polypeptide, using detection systems appropriate to the cells bearing the TL5 polypeptide. Inhibitors of activation are generally assayed in the presence of a known

agonist and the effect on activation by the agonist by the presence of the candidate compound is observed.

Alternatively, TL5 may be expressed as a soluble protein, including versions which fuse all or part of TL5 with a convenient partner peptide for which detection reagents are available, eg TL5-IgG fusions, and used in a solid state or solution phase binding assay. For example, the soluble TL5 can be used to detect agonist or antagonist binding directly through changes that can be detected experimentally, eg surface plasmon resonance, nuclear magnetic resonance spectrometry, sedimentation, calorimetry. The soluble TL5 can be used to detect agonist or antagonist binding directly by looking for competition of the candidate agonist or antagonist with a receptor whose binding can be detected. Receptor detection methods include antibody recognition, modification of the receptor via radioactive labeling, chemical modification (e.g., biotinylation), fusion to an epitope tag. Methods include ELISA based assays, immunoprecipitation and scintillation proximity. The receptor may also be obtained from natural sources (e.g., cells, cell membranes and cell supernatnants), but in these cases one would prefer to detect the binding of TL5 through methods including antibody recognition, modification of TL5 via radioactive labeling, chemical modification of TL5 (e.g., biotinylation), or fusion of TL5 to an epitope tag.

The TL5 cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of TL5 mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell associated levels of TL5 protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents which may inhibit or enhance the production of TL5 (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The TL5 protein may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. These include, but are not limited to, ligand binding and crosslinking assays in which the TL5 is labeled with a radioactive isotope (e.g., 125I), chemically modified (e.g., biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (cells, cell membranes, cell supernatants, tissue extracts, bodily fluids). Other methods include biophysical techniques such as surface plasmon resonance and spectroscopy. In addition to being used for purification and cloning of the receptor, these binding assays can be used to identify agonists and antagonists of TL5 which compete with the binding of TL5 to its receptors, if any. Standard methods for conducting screening assays are well understood in the art.

Examples of potential TL5 polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, enzymes, receptors,

etc., as the case may be, of the TL5 polypeptide, e.g., a fragment of the ligands, substrates, enzymes, receptors, etc.; or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for TL5 polypeptides; or compounds which decrease or enhance the production of TL5 polypeptides, which comprises:
(a) a TL5 polypeptide, preferably that of SEQ ID NO:2;
(b) a recombinant cell expressing a TL5 polypeptide, preferably that of SEQ ID NO:2;
(c) a cell membrane expressing a TL5 polypeptide; preferably that of SEQ ID NO: 2; or
(d) antibody to a TL5 polypeptide, preferably that of SEQ ID NO: 2.
It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Prophylactic and Therapeutic Methods

This invention provides methods of treating abnormal conditions such as, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, related to both an excess of and insufficient amounts of TL5 polypeptide activity.

If the activity of TL5 polypeptide is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit the function of the TL5 polypeptide, such as, for example, by blocking the binding of ligands, substrates, enzymes, receptors, etc., or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of TL5 polypeptides still capable of binding the ligand, substrate, enzymes, receptors, etc. in competition with endogenous TL5 polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the TL5 polypeptide.

In another approach, soluble forms of TL5 polypeptides still capable of binding the ligand in competition with endogenous TL5 polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the TL5 polypeptide.

In still another approach, expression of the gene encoding endogenous TL5 polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use

of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of TL5 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates TL5 polypeptide, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of TL5 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of TL5 polypeptides in combination with a suitable pharmaceutical carrier.

Formulation and Administration

Peptides, such as the soluble form of TL5 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 μg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Example 1

An EST (EST#1557446) with sequence similarity to human TNF was discovered in a commercial EST database. Analysis of the 530 nucleotide sequence of the partial cDNA indicated that it encoded an open reading frame for a novel member of the TNF superfamily and was named TL5. The predicted partial protein encoded by this cDNA is 147 amino acids long. This cDNA sequence was used to identify further ESTs which might encode the 5' end of the TL5 cDNA. One such EST encoded a complete open reading frame of 285 amino acids from a cDNA of 1093 nucleotides. The deduced protein encodes a type II membrane protein with a 46 amino acid cytoplasmic domain, an approximately 21 amino acid hydrophobic transmembrane spanning region, followed by a 218 amino acid extracellular domain which shares significant sequence identity with members of the TNF family and presumably encodes the receptor binding portion of the molecule.

A portion of the 3' untranslated sequence of the TL5 cDNA was identical to a human STS DNA sequence (Sequence tagged site) generated from the primer pair SHGC-36171. This fragment has been localized to chromosome 13, which is, therefore, where the gene for TL5 resides.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: SmithKline Beecham Corporation

(ii) TITLE OF THE INVENTION: A TNF homologue, TL5

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: SmithKline Beecham, Corporate Intellectual Property
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(C) CITY: Brentford
(D) STATE: Middlesex
(E) COUNTRY: United Kingdom
(F) ZIP: TW8 9EP

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: 03-DEC-1997
(C) CLASSIFICATION: UNKNOWN

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/041,797
(B) FILING DATE: 02-APR-1997

(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1093 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CACGAGAAAA TTCAGGATAA CTCTCCTGAG GGGTGAGCCA AGCCCTGCCA TGTAGTGCAC 60
GCAGGACATC AACAAACACA GATAACAGGA AATGATCCAT TCCCTGTGGT CACTTATTCT 120
AAAGGCCCCA ACCTTCAAAG TTCAAGTAGT GATATGGATG ACTCCACAGA AAGGGAGCAG 180
TCACGCCTTA CTTCTTGCCT TAAGAAAAGA GAAGAAATGA AACTGAAGGA GTGTGTTTCC 240
ATCCTCCCAC GGAAGGAAAG CCCCTCTGTC CGATCCTCCA AAGACGGAAA GCTGCTGGCT 300
GCAACCTTGC TGCTGGCACT GCTGTCTTGC TGCCTCACGG TGGTGTCTTT CTACCAGGTG 360
GCCGCCCTGC AAGGGGACCT GGCCAGCCTC CGGGCAGAGC TGCAGGGCCA CCACGCGGAG 420
AAGCTGCCAG CAGGAGCAGG AGCCCCCAAG GCCGGCCTGG AGGAAGCTCC AGCTGTCACC 480
GCGGGACTGA AAATCTTTGA ACCACCAGCT CCAGGAGAAG GCAACTCCAG TCAGAACAGC 540
AGAAATAAGC GTGCCGTTCA GGGTCCAGAA GAAACAGTCA CTCAAGACTG CTTGCAACTG 600
ATTGCAGACA GTGAAACACC AACTATACAA AAAGGATCTT ACACATTTGT TCCATGGCTT 660
CTCAGCTTTA AAAGGGGAAG TGCCCTAGAA GAAAAAGAGA ATAAAATATT GGTCAAAGAA 720
ACTGGTTACT TTTTTATATA TGGTCAGGTT TTATATACTG ATAAGACCTA CGCCATGGGA 780
CATCTAATTC AGAGGAAGAA GGTCCATGTC TTTGGGGATG AATTGAGTCT GGTGACTTTG 840
TTTCGATGTA TTCAAAATAT GCCTGAAACA CTACCCAATA ATTCCTGCTA TTCAGCTGGC 900
ATTGCAAAAC TGGAAGAAGG AGATGAACTC CAACTTGCAA TACCAAGAGA AAATGCACAA 960
ATATCACTGG ATGGAGATGT CACATTTTTT GGTGCATTGA AACTGCTGTG ACCTACTTAC 1020
ACCATGTCTG TAGCTATTTT CCTCCCTTTC TCTGTACCTC TAAGAAGAAA GAATCTAACT 1080
GAAAATACCA AAA 1093

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 285 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Asp Asp Ser Thr Glu Arg Glu Gln Ser Arg Leu Thr Ser Cys Leu
 1               5                  10                  15
Lys Lys Arg Glu Glu Met Lys Leu Lys Glu Cys Val Ser Ile Leu Pro
            20                  25                  30
Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly Lys Leu Leu
        35                  40                  45
Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys Leu Thr Val Val
    50                  55                  60
Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp Leu Ala Ser Leu Arg
65                  70                  75                  80
Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly
                85                  90                  95
Ala Pro Lys Ala Gly Leu Glu Glu Ala Pro Ala Val Thr Ala Gly Leu
            100                 105                 110
Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Asn
        115                 120                 125
Ser Arg Asn Lys Arg Ala Val Gln Gly Pro Glu Glu Thr Val Thr Gln
    130                 135                 140
Asp Cys Leu Gln Leu Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys
145                 150                 155                 160
Gly Ser Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser
                165                 170                 175
Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr
            180                 185                 190
Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met
        195                 200                 205
Gly His Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu
    210                 215                 220
Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu
225                 230                 235                 240
Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly
                245                 250                 255
Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu
            260                 265                 270
Asp Gly Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
        275                 280                 285
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 530 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGGAGAAGGC AACTCCAGTC AGAACAGCAG AAATAAGCGT GCCGTTCAGG GTCCAGAAGA 60
AACAGGATCT TACGAGACAT TTGTTCCATG GCTTCTCAGC TTTAAAAGGG GAAGTGCCCT 120
AGAAGAAAAA GAGAATAAAA TATTGGTCAA AGAAACTGGT TACTTTTTTA TATATGGTCA 180
GGTTTTATAT ACTGATAAGA CCTACGCCAT GGGACATCTA ATTCAGAGGA AGAAGGTCCA 240
TGTCTTTGGG GATGAATTGA GTCTGGTGAC TTTGTTTCGA TGTATTCAAA ATATGCCTGA 300
AACACTACCC AATAATTCCT GCTATTCAGC TGGCATTGCA AAACTGGAAG AAGGAGATGA 360
ACTCCAACTT GCAATACCAA GAGAAAATGC ACAAATATCA CTGGATGGAG ATGTCACATT 420
TTTTGGTGCA TTGAAACTGC TGTGACCTAC TTACACCATG TCTGTAGCTA TTTTCCTCCC 480
TTTCTCTGTA CCTCTAAGAA GAAAGAATCT AACTGAAAAT ACCAAAAAAA 530

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 147 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gly Glu Gly Asn Ser Ser Gln Asn Ser Arg Asn Lys Arg Ala Val Gln
1 5 10 15
Gly Pro Glu Glu Thr Gly Ser Tyr Glu Thr Phe Val Pro Trp Leu Leu
20 25 30
Ser Phe Lys Arg Gly Ser Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu
35 40 45
Val Lys Glu Thr Gly Tyr Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr
50 55 60
Asp Lys Thr Tyr Ala Met Gly His Leu Ile Gln Arg Lys Lys Val His
65 70 75 80

```
Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln
                85                  90                  95
Asn Met Pro Glu Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile
            100                 105                 110
Ala Lys Leu Glu Glu Gly Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu
        115                 120                 125
Asn Ala Gln Ile Ser Leu Asp Gly Asp Val Thr Phe Phe Gly Ala Leu
    130                 135                 140
Lys Leu Leu
145
```

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the TL5 polypeptide of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.

2. The polynucleotide of claim 1 wherein said polynucleotide comprises the nucleotide sequence contained in SEQ ID NO:1 encoding the TL5 polypeptide of SEQ ID NO2.

3. The polynucleotide of claim 1 wherein said polynucleotide comprises a nucleotide sequence that is at least 80% identical to that of SEQ ID NO: 1 over its entire length.

4. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.

5. The polynucleotide of claim 1 which is DNA or RNA.

6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a TL5 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.

7. A host cell comprising the expression system of claim 6.

8. A process for producing a TL5 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.

9. A process for producing a cell which produces a TL5 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a TL5 polypeptide.

10. A TL5 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.

11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.

12. An antibody immunospecific for the TL5 polypeptide of claim 10.

13. A method for the treatment of a subject in need of enhanced activity or expression of TL5 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an agonist to said polypeptide; and/or

(b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the TL5 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said polypeptide activity *in vivo*.

14. A method for the treatment of a subject having need to inhibit activity or expression of TL5 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an antagonist to said polypeptide; and/or

(b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said polypeptide; and/or

(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said polypeptide for its ligand, substrate , or receptor.

15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of TL5 polypeptide of claim 10 in a subject comprising:

(a) determining the presence or absence of a mutation in the nucleotide sequence encoding said TL5 polypeptide in the genome of said subject; and/or.

(b) analyzing for the presence or amount of the TL5 polypeptide expression in a sample derived from said subject.

16. A method for identifying compounds which inhibit (antagonize) or agonize the TL5 polypeptide of claim 10 which comprises:

(a) contacting a candidate compound with cells which express the TL5 polypeptide (or cell membrane expressing TL5 polypeptide) or respond to TL5 polypeptide; and

(b) observing the binding, or stimulation or inhibition of a functional response; or comparing the ability of the cells (or cell membrane) which were contacted with the candidate compounds with the same cells which were not contacted for TL5 polypeptide activity.

17. An agonist identified by the method of claim 16.

18. An antagonist identified by the method of claim 16.

19. A recombinant host cell produced by a method of Claim 9 or a membrane thereof expressing a TL5 polypeptide.

6

ABSTRACT OF THE DISCLOSURE

TL5 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing TL5 polypeptides and polynucleotides in the design of protocols for the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others, and diagnostic assays for such conditions.

25-11-1998 EP98309632.2 1001P

Antrag auf Erteilung eines europäischen Patents / Request for grant of a European patent / Requête en délivrance d'un brevet européen

Bestätigung einer bereits durch Telekopie (Telefax) eingereichten Anmeldung / Confirmation of an application already filed by facsimile / Confirmation d'une demande déjà déposée par télécopie
Wenn ja, Datum der Übermittlung der Telekopie und Name der Einreichungsbehörde / If yes, facsimile date and name of the authority with which the documents were filed / Si oui, date d'envoi de la télécopie et nom de l'autorité de dépôt

☐ Ja / Yes / Oui

Datum / Date | Behörde / Authority / Autorité

Nur für amtlichen Gebrauch / For official use only / Cadre réservé à l'administration

Anmeldenummer / Application No. / N° de la demande	MKEY	1	98309632.2
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC	2	25 NOVEMBER 1998
Tag des Eingangs beim EPA (Regel 24(4)) / Date of receipt at EPO (Rule 24(4)) / Date de réception à l'OEB (règle 24(4))	RENA	3	EPO DG 1 09. 12. 1998
Anmeldetag / Date of filing / Date de dépôt		4	

Tabulatoren-Positionen / Tabulation marks / Arrêts de tabulation

Es wird die Erteilung eines europäischen Patents und gemäß Artikel 94 die Prüfung der Anmeldung beantragt / Grant of a European patent, and examination of the application under Article 94, are hereby requested / Il est demandé la délivrance d'un brevet européen et, conformément à l'article 94, l'examen de la demande — EXAM 4 — 5 ☒

Prüfungsantrag in einer zugelassenen Nichtamtssprache (siehe Merkblatt II, 5): / Request for examination in an admissible non-EPO language (see Notes II,5): / Requête en examen dans une langue non officielle autorisée (voir notice II,5):

Zeichen des Anmelders oder Vertreters (max. 15 Positionen) / **Applicant's or representative's reference** (maximum 15 spaces) / **Référence du demandeur ou du mandataire** (max. 15 caractères ou espaces) — AREF — 6: X-11834

ANMELDER / APPLICANT / DEMANDEUR
Name / Nom — 7: ELI LILLY AND COMPANY

Anschrift / Address / Adresse — 8:
LILLY CORPORATE CENTER,
INDIANAPOLIS,
INDIANA 46285,
UNITED STATES OF AMERICA

APPR 01 # 120191427
DEST

Zustellanschrift / Address for correspondence / Adresse pour la correspondance — 9

PADR

Staat des Wohnsitzes oder Sitzes / State of residence or of principal place of business / Etat du domicile ou du siège — 10: UNITED STATES OF AMERICA

Staatsangehörigkeit / Nationality / Nationalité — 11: UNITED STATES OF AMERICA

Telefon / Telephone / Téléphone — 12

Telex / Télex | **Telefax / Fax / Téléfax** — 13

Weitere(r) Anmelder auf Zusatzblatt / Additional applicant(s) on additional sheet / Autre(s) demandeur(s) sur feuille additionnelle — 14 ☐

VERTRETER / REPRESENTATIVE / MANDATAIRE:
Name / Nom — 15: A. M. DENHOLM

(Nur einen Vertreter angeben, der in das europäische Patentregister eingetragen und an den zugestellt wird / Name **only one** representative, who is to be listed in the Register of European Patents and to whom notification is to be made / N'indiquer qu'**un seul** mandataire, qui sera inscrit au Registre européen des brevets et auquel signification sera faite)

FREP 01 | 101781621218 # | | | | | # /#

Geschäftsanschrift / Address of place of business / Adresse professionnelle — 16:
LILLY RESEARCH CENTRE,
ERL WOOD MANOR,
WINDLESHAM,
SURREY GU20 6PH
UNITED KINGDOM

Telefon / Telephone / Téléphone — 17: 01276 853443

Telex / Télex | **Telefax / Fax / Téléfax** — 18: 01276 853306

Weitere(r) Vertreter auf Zusatzblatt / Additional representative(s) on additional sheet / Autre(s) mandataire(s) sur feuille additionnelle — 19 ☐

TRAN | | FILL | E | N

X-11834

Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

Vollmacht / Authorisation / Pouvoir:

ist beigefügt / is enclosed / ci-joint — 20 ☐

ist registriert unter Nummer / has been registered under No. / a été enregistré sous le n° — GENA — 21 ∞3070.0 — Nummer / Number / Numéro

ERFINDER / INVENTOR / INVENTEUR: — INVT 20 # #

Anmelder ist (sind) alleinige(r) Erfinder / The applicant(s) is (are) the sole inventor(s) / Le(s) demandeur(s) est (sont) le (les) seul(s) inventeur(s) — 22 ☐

Erfindernennung auf gesondertem Schriftstück / Designation of inventor attached / Voir la désignation de l'inventeur ci-jointe — 23 X

BEZEICHNUNG DER ERFINDUNG / TITLE OF INVENTION / TITRE DE L'INVENTION: — 24

TNF LIGAND FAMILY GENE

TIDE | TIEN | TIFR

PRIORITÄTSERKLÄRUNG / DECLARATION OF PRIORITY / DECLARATION DE PRIORITE — PRIO — 25

	Staat / State / Etat	Anmeldetag / Filing date / Date de dépôt	Aktenzeichen / Application No. / N° de la demande
1	USA	26 NOV 97	60/066,577
	USA	11 AUG 98	60/096,173
2			
3			
4			

01 # # #

02 # # #

03 # # #

04 # # #

Weitere Prioritätserklärung(en) auf Zusatzblatt / Additional declaration(s) of priority on additional sheet / Autre(s) déclaration(s) de priorité sur feuille additionnelle ☐

BIOLOGISCHES MATERIAL

Die Erfindung betrifft biologisches Material oder seine Verwendung, das nach Regel 28 hinterlegt worden ist.

BIOLOGICAL MATERIAL

The invention relates to and/or uses biological material deposited under Rule 28.

26 ☐ MATIERE BIOLOGIQUE

L'invention concerne et/ou utilise la matière biologique, déposée conformément à la règle 28.

BIOM 1 # # #

Die Angaben nach Regel 28(1) c) sind in den technischen Anmeldungsunterlagen enthalten auf / The particulars referred to in Rule 28(1) (c) are given in the technical documents in the application on / Les indications visées à la règle 28(1) c) figurent dans les pièces techniques de la demande à la /aux — 27 Seite(n) / page(s) | Zeile(n) / line(s) / ligne(s)

werden später mitgeteilt / will be submitted later / seront communiquées ultérieurement — 27a ☐

Die Empfangsbescheinigung(en) der Hinterlegungsstelle ist (sind) beigefügt / The receipt(s) of deposit issued by the depositary institution is (are) enclosed / Le(s) récépissé(s) de dépôt délivré(s) par l'autorité de dépôt est (sont) ci-joint(s) — 28 ☐

wird (werden) nachgereicht / will be filed later / sera (seront) produit(s) ultérieurement — 28a ☐

Verzicht auf die Verpflichtung des Antragstellers nach Regel 28(3) auf gesondertem Schriftstück / Waiver of the right to an undertaking from the requester pursuant to Rule 28(3) attached / Renonciation, sur document distinct, à l'engagement du requérant au titre de la règle 28(3) — 29 ☐

X-11834

Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

Falls das biologische Material nicht vom Anmelder, sondern von einem Dritten hinterlegt wurde: / Where the biological material has been deposited by a person other than the applicant: / Lorsque la matière biologique a été déposée par une personne autre que le demandeur:

30 Name und Anschrift des Hinterlegers / Name and address of depositor / Nom et adresse du déposant :

Ermächtigung nach Regel 28(1)d) / Authorisation under Rule 28(1)(d) / Autorisation en vertu de la règle 28(1)d)

ist beigefügt / is enclosed / ci-jointe 30a ☐

wird nachgereicht / will be filed later / sera produite ultérieurement 30b ☐

NUCLEOTID-UND AMINOSÄURESEQUENZEN / NUCLEOTIDE AND AMINO ACID SEQUENCES / SEQUENCES DE NUCLEOTIDES ET D'ACIDES AMINES SEQL (1) 31

Die Beschreibung enthält ein Sequenzprotokoll nach Regel 27a(1) / The description contains a sequence listing in accordance with Rule 27a(1) / La description contient une liste de séquences selon la règle 27bis(1) X

Der vorgeschriebene maschinenlesbare Datenträger ist beigefügt / The prescribed machine readable data carrier is enclosed / Le support de données prescrit déchiffrable par machine est annexé X

Es wird hiermit erklärt, daß die auf dem Datenträger gespeicherte Information mit dem schriftlichen Sequenzprotokoll übereinstimmt (Regel 27a(2)) / It is hereby stated that the information recorded on the data carrier is identical to the written sequence listing (Rule 27a(2)) /Il est déclaré par la présente que l'information figurant sur le support de données est identique à celle que contient la liste de séquences écrite (règle 27bis (2)) X

BENENNUNG DER VERTRAGSSTAATEN UND ERKLÄRUNGEN HIERZU

1. Hiermit werden sämtliche Vertragsstaaten des EPÜ benannt, die bei Einreichung dieser Anmeldung dem EPÜ angehören.
2. Der Anmelder beabsichtigt derzeit, Benennungsgebühren für die nachfolgend angekreuzten Vertragsstaaten zu entrichten:

DESIGNATION OF THE CONTRACTING STATES AND ASSOCIATED DECLARATIONS

1. All States which are Contracting States to the EPC at the filing of this application are hereby designated.
2. The applicant currently intends to pay designation fees for the States marked below with a cross:

32

DESIGNATION D'ETATS CONTRACTANTS ET DECLARATIONS A CE PROPOS

☒ 1. Sont désignés tous les Etats qui sont des Etats contractants de la CBE à la date du dépôt de la présente demande.

2. Le demandeur se propose actuellement de payer des taxes de désignation pour les Etats cochés ci-dessous :

DEST

X	AT	Österreich / Austria / Autriche	X	GB	Vereinigtes Königreich / United Kingdom / Royaume-Uni
X	BE	Belgien / Belgium / Belgique	X	GR	Griechenland / Greece / Grèce
X	CH/LI	Schweiz und Liechtenstein / Switzerland and Liechtenstein / Suisse et Liechtenstein	X	IE	Irland / Ireland / Irlande
			X	IT	Italien / Italy / Italie
X	DE	Deutschland / Germany / Allemagne		LU	Luxemburg / Luxembourg / Luxembourg
X	DK	Dänemark / Denmark / Danemark		MC	Monaco / Monaco / Monaco
X	ES	Spanien / Spain / Espagne	X	NL	Niederlande / Netherlands / Pays-Bas
X	FI	Finnland / Finland / Finlande	X	PT	Portugal / Portugal / Portugal
X	FR	Frankreich / France / France	X	SE	Schweden / Sweden / Suède
X	~~CY~~	~~Cyprus~~		—	
	—			—	

(Platz für Vertragsstaaten, für die das EPÜ nach Drucklegung dieses Formblatts in Kraft tritt / Space for Contracting States for which the EPC enters into force after this form has been printed / Prévu pour des Etats contractants à l'égard desquels la CBE entrera en vigueur après l'impression du présent formulaire)

Es wird beantragt, für die unter Nr. 2 nicht angekreuzten Vertragsstaaten von der Zustellung von Mitteilungen nach Regel 85a (1) und Regel 69 (1) abzusehen.
Ist ein **automatischer Abbuchungsauftrag** erteilt worden (Feld 43), so wird beantragt, bei Ablauf der Grundfrist nach Artikel 79 (2) Benennungsgebühren nur für die unter Nr. 2 angekreuzten Vertragsstaaten abzubuchen.

It is requested that no communications under Rule 85a(1) and Rule 69(1) be notified concerning the Contracting States not marked with a cross under No. 2.
If an **automatic debit order** has been given (section 43), it is requested that, when the basic period specified in Art. 79(2) expires, designation fees be debited only for the Contracting States marked with a cross under No. 2.

☒ Prière de ne pas procéder à la signification des notifications prévues par les règles 85bis(1) et 69(1) pour les Etats contractants n'ayant pas été cochés au n° 2.
Si un **ordre de prélèvement automatique** a été donné (rubrique 43), prière de ne prélever à l'expiration des délais de base tels que définis à l'article 79(2) que les taxes de désignation pour les Etats contractants cochés au n° 2.

X-11834

Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

Verschiedene Anmelder für verschiedene Vertragsstaaten / Different applicants for different Contracting States / Différents demandeurs pour différents Etats contractants

APPR 02 # #

33 Name(n) des (der) Anmelder(s) und benannte Vertragsstaaten / Name(s) of applicant(s) and designated Contracting States / Nom(s) du (des) demandeur(s) et des Etats contractants désignés

34

ERSTRECKUNG DES EUROPÄISCHEN PATENTS

Diese Anmeldung gilt als Antrag, die europäische Patentanmeldung und das darauf erteilte europäische Patent auf alle Nicht-Vertragsstaaten des EPÜ zu erstrecken, mit denen am Tag ihrer Einreichung „Erstreckungsabkommen" bestehen (Derzeit: Albanien, Litauen, Lettland, Rumänien, Slowenien). Die Erstreckung wird jedoch nur wirksam, wenn die vorgeschriebene Erstreckungsgebühr entrichtet wird.

EXTENSION OF THE EUROPEAN PATENT

This application is deemed to be a request to extend the European patent application and the European patent granted in respect of it to all non-Contracting States to the EPC with which "extension agreements" exist on the date on which the application is filed (Present situation: Albania, Lithuania, Latvia, Romania, Slovenia). However, the extension only takes effect if the prescribed extension fee is paid.

EXTENSION DES EFFETS DU BREVET EUROPEEN

La présente demande est réputée constituer une requête en extension des effets de la demande de brevet européen et du brevet européen délivré sur la base de cette demande à tous les Etats non parties à la CBE avec lesquels il existe un «accord d'extension» à la date du dépôt de la demande (Situation actuelle : Albanie, Lituanie, Lettonie, Roumanie, Slovénie). Toutefois l'extension ne produit ses effets que s'il est acquitté la taxe d'extension prescrite.

EXPT

Der Anmelder beabsichtigt derzeit, die Erstreckungsgebühr für die nachfolgend angekreuzten Staaten zu entrichten: / The applicant currently intends to pay the extension fee for the States marked below with a cross: / Le demandeur se propose actuellement d'acquitter la taxe d'extension pour les Etats dont le nom est coché ci-après :

Albanien / Albania / Albanie	AL	☐
Litauen / Lithuania / Lituanie	LT	☐
Lettland / Latvia / Lettonie	LV	☐
Rumänien / Romania / Roumanie	RO	☐
Slowenien / Slovenia / Slovénie	SI	☐
		☐
		☐
		☐

(Platz für Staaten, mit denen nach Drucklegung dieses Formblatts „Erstreckungsabkommen" in Kraft treten) / (Space for States with which "extension agreements" enter into force after this form has been printed) / (Prévu pour des Etats à l'égard desquels des «accords d'extension» entreront en vigueur après l'impression du présent formulaire)

Die Anmeldung ist eine Teilanmeldung / The application is a divisional application / La présente demande constitue une demande divisionnaire

DFIL 9 #

PANR #

35 ☐ Nummer der früheren Anmeldung / No. of earlier application / Numéro de la demande initiale

Es handelt sich um eine Anmeldung nach Art. 61(1)b) / The application is an Art. 61(1)(b) application / La présente demande constitue une demande selon l'article 61(1)b)

DFIL 9 #

EANR #

36 ☐ Nummer der früheren Anmeldung / No. of earlier application / Numéro de la demande initiale

Patentansprüche / Claims / Revendications CLMS

37 10 Zahl der Patentansprüche / Number of claims / Nombre de revendications

Zur Veröffentlichung mit der Zusammenfassung wird vorgeschlagen Abbildung Nr. / With the abstract it is proposed to publish figure No. / Il est proposé de publier avec l'abrégé la figure n° DRAW (2)

39 Nummer / Number / Numéro

Zusätzliche Abschrift(en) der im europäischen Recherchenbericht angeführten Schriftstücke wird (werden) beantragt / Additional copy(ies) of the documents cited in the European search report is (are) requested / Prière de fournir une (des) copie(s) supplémentaire(s) des documents cités dans le rapport de recherche européenne

ASOC

40 1

Anzahl der **zusätzlichen** Sätze von Abschriften
Number of **additional** sets of copies
Nombre de jeux **supplémentaires** de copies

Es wird die Rückerstattung der Recherchengebühr gemäß Art. 10 GebO beantragt / Refund of the search fee is requested pursuant to Article 10 of the Rules relating to Fees / Le remboursement de la taxe de recherche est demandé en vertu de l'article 10 du règlement relatif aux taxes

41 ☐

Eine Kopie des Recherchenberichts ist beigefügt / A copy of the search report is attached / Une copie du rapport de recherche est jointe

42 ☐

AUTOMATISCHER ABBUCHUNGSAUFTRAG *(nur möglich für Inhaber von beim EPA geführten laufenden Konten)*
AUTOMATIC DEBIT ORDER *(for EPO deposit account holders only)*
ORDRE DE PRELEVEMENT AUTOMATIQUE *(uniquement possible pour les titulaires de comptes courants ouverts auprès de l'OEB)*

Das Europäische Patentamt wird hiermit beauftragt, fällig werdende Gebühren und Auslagen nach Maßgabe der Vorschriften über das automatische Abbuchungsverfahren vom nebenstehenden laufenden Konto abzubuchen / The European Patent Office is hereby authorised, under the Arrangements for the automatic debiting procedure, to debit from the deposit account opposite any fees and costs falling due / Par la présente, il est demandé à l'Office européen des brevets de prélever du compte courant ci-contre les taxes et frais venant à échéance, conformément à la réglementation relative au prélèvement automatique

DECA

FÜR AUTOMATISCHEN ABBUCHUNGSAUFTRAG:
FOR AUTOMATIC DEBIT ORDER:
POUR L'ORDRE DE PRELEVEMENT AUTOMATIQUE:

Nummer des laufenden Kontos / Deposit account number / Numéro du compte courant

Name des Kontoinhabers / Account holder's name / Nom du titulaire du compte

43

Eventuelle **RÜCKZAHLUNGEN** auf das nebenstehende beim EPA geführte laufende Konto / **REIMBURSEMENT**, if any, to EPO deposit account opposite / **REMBOURSEMENTS** éventuels à effectuer sur le compte courant ci-contre ouvert auprès de l'OEB

DEPA

Nummer des laufenden Kontos / Deposit account number / Numéro du compte courant

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44 28050027 ELI LILLY AND COMPANY LIMITED

Die vorgeschriebene Liste über die diesem Antrag beigefügten Unterlagen ergibt sich aus der vorbereiteten Empfangsbescheinigung (Seite 6 dieses Antrages)

The prescribed list of documents enclosed with this request is shown on the prepared receipt (page 6 of this request)

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La liste prescrite des documents joints à cette requête figure sur le récépissé préétabli (page 6 de la présente requête)

Unterschrift(en) des (der) Anmelder(s) oder Vertreter(s) / Signature(s) of applicant(s) or representative(s) / Signature(s) du (des) demandeur(s) ou du (des) mandataire(s)

46

Für Angestellte nach Artikel 133(3) Satz 1 mit allgemeiner Vollmacht / For employees under Article 133(3), 1st sentence, having a general authorisation / Pour les employés mentionnés à l'article 133(3), 1ère phrase, munis d'un pouvoir général
Nr. / No. / n° :

Ort / Place / Lieu WINDLESHAM, SURREY, U.K.

Datum / Date 23RD NOVEMBER 1998

Mr. A. M. DENHOLM eA307
EUROPEAN PATENT ATTORNEY

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X-11834

Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

Empfangsbescheinigung / Receipt for documents / Récépissé de documents

(Liste der diesem Antrag beigefügten Unterlagen) (Checklist of enclosed documents) (Liste des documents annexés à la présente requête)

Es wird hiermit der Empfang der unten bezeichneten Dokumente bescheinigt / Receipt of the documents indicated below is hereby acknowledged / Nous attestons le dépôt des documents désignés ci-dessous

Wird im Falle der Einreichung der europäischen Patentanmeldung bei einer nationalen Behörde diese Empfangsbescheinigung vom Europäischen Patentamt übersandt, so ist sie als Mitteilung gemäß Regel 24(4) anzusehen (siehe Feld RENA). **Nach Erhalt der Mitteilung nach Regel 24(4) sind alle weiteren Unterlagen, die die Anmeldung betreffen, nur noch unmittelbar beim EPA einzureichen.** / If this receipt is issued by the European Patent Office and the European patent application was filed with a national authority it serves as a communication under Rule 24(4) (see Section RENA). **Once the communication under Rule 24(4) has been received, all further documents relating to the application must be sent directly to the European Patent Office.** / Si, en cas de dépôt de la demande de brevet européen auprès d'un service national, l'Office européen des brevets délivre le présent récépissé de documents, ce récépissé est réputé être la notification visée à la règle 24(4). **Dès que la notification visée à la règle 24(4) a été reçue, tous les autres documents relatifs à la demande doivent être adressés directement à l'OEB.**

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THE PATENT OFFICE
25 NOV 1998
LONDON

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Anmeldenummer / Application No. / N° de la demande			98309632.2
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC		25 NOVEMBER 1998
Zeichen des Anmelders/Vertreters / Applicant's/ Representative's ref. / Référence du demandeur ou du mandataire	AREF		X-11834
Nur nach Einreichung der Anmeldung bei einer nationalen Behörde: / Only after filing of the application with a national authority: / Seulement après le dépôt de la demande auprès d'un service national:			
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A.	**Anmeldungsunterlagen und Prioritätsbeleg(e) / Application documents and priority document(s) / Pièces de la demande et document(s) de priorité**	47			
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3.	Zeichnung(en) / Drawing(s) / Dessin(s)	DRAW 1 #			
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6.	Prioritätsbeleg(e) / Priority document(s) / Document(s) de priorité				
7.	Übersetzung des (der) Prioritätsbelegs(belege) / Translation of priority document(s) / Traduction du (des) document(s) de priorité				
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1.	Einzelvollmacht / Specific authorisation / Pouvoir particulier				
2.	Allgemeine Vollmacht / General authorisation / Pouvoir général				
3.	Erfindernennung / Designation of inventor / Désignation de l'inventeur		X		
4.	Früherer Recherchenbericht / Earlier search report / Rapport de recherche antérieure				
5.	Gebührenzahlungsvordruck (EPA Form 1010) / Voucher for the settlement of fees (EPO Form 1010) / Bordereau de règlement de taxes (OEB Form 1010)		X		
6.	Scheck *(ausgeschlossen bei Einreichung bei den nationalen Behörden)* / Chèque *(not when filing with national authorities)* / Chèque *(pas de chèque en cas de dépôt auprès des services nationaux)*				
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8.	Zusatzblatt / Additional sheet / Feuille additionnelle				
9.	Sonstige Unterlagen (bitte hier spezifizieren) / Other (please specify here) / Autres documents (veuillez préciser ici)				
C.	**Kopien dieser Empfangsbescheinigung / Copies of this receipt for documents / Copies du présent récépissé de documents**	49	3	Anzahl der Kopien / Number of copies / Nombre de copies	

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(falls Anmelder nicht oder nicht allein der Erfinder ist) / (where the applicant is not the inventor or is not the sole inventor) / (si le demandeur n'est pas l'inventeur ou l'unique inventeur)

Nr. der Anmeldung oder, falls noch nicht bekannt, Bezeichnung der Erfindung
Application N° or, if not yet known, title of the invention
N° de la demande ou, si ce dernier n'est pas encore connu, titre de l'invention

TNF LIGAND FAMILY GENE

Zeichen des Anmelders oder Vertreters
Applicant's or representative's reference
Référence du demandeur ou du mandataire
(max. 15 Positionen / max. 15 spaces / 15 caractères au maximum)

X-11834

In Sachen der obenbezeichneten europäischen Patentanmeldung nennt (nennen) der (die) Unterzeichnete(n)[1]
In respect of the above European patent application I (we), the undersigned [1]
En ce qui concerne la demande de brevet européen susmentionnée le (s) soussigné(s)[1]

A. M. DENHOLM

als Erfinder[2]:
do hereby designate as inventor(s)[2]:
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☐ (Weitere Erfinder sind auf einem gesonderten Blatt angegeben) / (Additional inventors indicated on supplementary sheet) / (les autres inventeurs sont mentionnés sur une feuille supplémentaire).

Der (Die) Anmelder hat (haben) das Recht auf das europäische Patent erlangt[3]
The applicant(s) has (have) acquired the right to the European patent[3]
Le(s) demandeur(s) a (ont) acquis le droit au brevet européen[3]

☒ gemäß Vertrag vom 11th February 1998 & 11th November 1998
under an agreement dated
par contrat en date du

☐ als Arbeitgeber / as employer(s) / en qualité d'employeur(s)

☐ durch Erbfolge / as successor(s) in title / par transfert successoral

Ort/Place/Lieu WINDLESHAM, SURREY, U.K. Datum/Date 23RD NOVEMBER 1998

Unterschrift(en) des (der) Anmelder(s) oder Vertreter(s) /
Signature(s) of applicant(s) or representative(s) /
Signature(s) du (des) demandeur(s) ou du (des) mandataire(s)

A. M. DENHOLM
EUROPEAN PATENT ATTORNEY
9A307

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Fußnoten befinden sich auf der Rückseite / Footnotes overleaf / Le texte des renvois figure au verso

TNF LIGAND FAMILY GENE

This application claims the benefit of U.S Provisional Application No. 60/066,577, filed November 26, 1997, and U.S. Provisional Application No. 60/096,173, filed August 11, 1998.

This invention relates to recombinant DNA technology. In particular the invention pertains to a TNF ligand family gene, and its encoded protein. Also contemplated are methods for identifying compounds that bind said ligand, and methods for treating oncological disease in mammals.

Apoptosis (*i.e.* programmed cell death), is a process fundamental to the normal development and homeostasis of multicellular organisms. Deregulation of programmed cell death leads to a number of human diseases, including cancer, neurodegenerative disorders, and acquired immunodeficiency syndrome. The cell death machinery comprises effectors, activators, and negative regulators. Certain cytokines of the tumor necrosis factor (TNF) ligand family and their cognate receptors, including TNFR-1 and Fas (also known as Apo-1 or CD95), are classic triggers of the suicide response. TNF is the prototypic member of an emerging family of cytokines that function as prominent mediators of immune regulation and the inflammatory response. Eight other members of the TNF family are known, including lymphotoxin (Ltα, TNFβ), lymphotoxin β (LTβ), and ligands for CD40, CD30, CD27, OX40, 4-1BB, and Fas (APO-1). With one

exception, all TNF ligands are type II membrane proteins, having homology at the C-terminal end. The exception, LTα, appears to be a secreted protein that also exists as a cell surface-associated member, termed LTβ. In addition, a proteolytically processed soluble form of TNF has been studied.

The TNF ligands interact with a parallel family of some twelve homologous receptors, characterized by cysteine-rich psuedorepeats in the extracellular region. As with the TNF ligands, the TNF receptors are variably expressed in a variety of cell types, including B cells, T cells, dendritic cells, and macrophages.

TNA and Fas ligand (also known as Apo-1L or CD95L) induce apoptosis by binding to receptors TNFR-1 and Fas. These receptors contain a domain (termed "death domain") that mediates the assembly of a signaling complex, leading to the recruitment of pro-apoptotic proteases.

Another member of the TNF ligand family, termed TRAIL (also known as Apo-2L), has been identified. Like the Fas ligand (FasL), TRAIL induces rapid apoptosis in transformed cell lines of diverse origin. Unlike FasL, whose transcripts are predominantly restricted to stimulated T cells, TRAIL expression is detected in many normal human tissues. This suggests that TRAIL is a member of the TNF ligand family that has marked pro-apoptotic potential for transformed cells. However, the inability of TRAIL to bind TNFR-1, Fas, or the recently identified death domain-containing receptor DR3 (also called Ws1-1, Apo-3, and TRAMP) suggests that TRAIL may interact with a yet unknown member of the TNF-receptor family.

The present invention provides isolated nucleic acid molecules and novel members of the TNF ligand family, termed herein "TRAILLK-2". Having the cloned TRAILLK-2 gene enables the production of recombinant protein, the isolation of orthologous genes from other organisms, and/or paralogous genes from the same organism, chromosome mapping studies, and the implementation of large scale screens to identify compounds that bind said receptor protein, as a means to identify potential pharmaceutical compounds for modulating the biological activity thereof. The proteins and peptides described herein are also useful therapeutic agents *per se* and for developing new compounds for treating cancer, and, among other things, as feed additives.

In one embodiment the present invention relates to an isolated nucleic acid molecule encoding TRAILLK-2 protein.

In another embodiment, the invention relates to a nucleic acid molecule comprising the nucleotide sequence identified as SEQ ID NO:1.

In another embodiment, the present invention relates to a nucleic acid that encodes SEQ ID NO:2, or fragment thereof.

In another embodiment, the present invention relates to a nucleic acid that is at least 75% identical to a nucleic acid that encodes SEQ ID NO:2, or fragment thereof.

In another embodiment, the present invention relates to a nucleic acid that hybridizes to SEQ ID NO:1 under high stringency conditions and encodes a protein that is capable of inducing apoptosis, and/or in treating cancer, and/or in

preventing or inhibiting cancerous tumor growth, and/or in causing shrinkage of a cancerous tumor.

In another embodiment, the present invention relates to a nucleic acid that hybridizes to SEQ ID NO:1 under low stringency conditions and encodes a protein that is capable of inducing apoptosis and/or in treating cancer, and/or in preventing or inhibiting cancerous tumor growth, and/or in causing shrinkage of a cancerous tumor.

In another embodiment the present invention relates to an isolated protein molecule, or functional fragment thereof, wherein said protein molecule comprises the sequence identified as SEQ ID NO:2.

In yet another embodiment, the present invention relates to a recombinant DNA vector that incorporates the TRAILLK-2 gene (SEQ ID NO:1) in operable-linkage to gene expression sequences, enabling the gene to be transcribed and translated in a host cell.

In still another embodiment the present invention relates to host cells that have been transformed or transfected with the cloned TRAILLK-2 gene such that said gene is expressed in the host cell.

This invention also provides a method of determining whether a nucleic acid sequence of the present invention, or fragment thereof, is present within a nucleic acid-containing sample, comprising contacting the sample under suitable hybridization conditions with a nucleic acid probe of the present invention.

In a still further embodiment, the present invention relates to a method for treating cancer, and/or in

preventing or inhibiting cancerous tumor growth, and/or in causing shrinkage of a cancerous tumor.

Definitions

"Apoptosis" refers to the phenomenon of programmed cell death that is fundamental to the normal development and homeostasis of multicellular organisms. Deregulation of apoptosis leads to a number of human diseases, including cancer, neurodegenerative disorders, and AIDS. Certain cytokines of the TNF ligand family are triggers of apoptosis.

The terms "complementary" or "complementarity" as used herein refer to the capacity of purine and pyrimidine nucleotides to associate through hydrogen bonding to form double stranded nucleic acid molecules. The following base pairs are related by complementarity: guanine and cytosine; adenine and thymine; and adenine and uracil. As used herein, "complementary" means that the aforementioned relationship applies to substantially all base pairs comprising two single-stranded nucleic acid molecules over the entire length of said molecules. "Partially complementary" refers to the aforementioned relationship in which one of two single-stranded nucleic acid molecules is shorter in length than the other such that a portion of one of the molecules remains single-stranded.

"Conservative substitution" or "conservative amino acid substitution" refers to a replacement of one or more amino acid residue(s) in a protein or peptide as stipulated in Table 1.

"Fragment thereof" refers to a fragment, piece, or sub-region of a nucleic acid or protein molecule whose sequence is disclosed herein, such that the fragment comprises 5 or more amino acids, or 10 or more nucleotides that are contiguous in the parent protein or nucleic acid molecule. Fragment thereof may or may not retain biological activity. For example, a fragment of a protein disclosed herein could be used as an antigen to raise a specific antibody against the parent protein molecule. When referring to a nucleic acid molecule, "fragment thereof" refers to 10 or more contiguous nucleotides, derived from the parent nucleic acid, and also, owing to the genetic code, to the complementary sequence. For example if the fragment entails the sequence 5'-AGCTAG-3', then "fragment thereof" would also include the complementary sequence, 3'-TCGATC-5'.

The term "fusion protein" denotes a hybrid protein molecule not found in nature comprising a translational fusion or enzymatic fusion in which two or more different proteins or fragments thereof are covalently linked on a single polypeptide chain.

"Functional fragment" or "functionally equivalent fragment", as used herein, refers to a region, or fragment of a full length protein, or sequence of amino acids that, for example, comprises an active site, or any other conserved motif, relating to biological function. Functional fragments are capable of providing a substantially similar biological activity as a full length protein disclosed herein, *in vivo* or *in vitro*, *viz.* the capacity to promote apoptosis. Functional fragments may be produced by cloning

technology, or as the natural products of alternative splicing mechanisms.

"Host cell" refers to any eucaryotic or procaryotic cell that is suitable for propagating and/or expressing a cloned gene contained on a vector that is introduced into said host cell by, for example, transformation or transfection, or the like.

TRAILLK-2 refers to a gene (SEQ ID NO:1) and a protein (SEQ ID NO:2) or peptide encoded thereby. TRAILLK-2 is a member of the family of TNF ligands. This family mediates a variety of biological effects including apoptosis, especially in tumor cells, and induction of the immune response.

The term "homolog" or "homologous" describes the relationship between different nucleic acid molecules or amino acid sequences in which said sequences or molecules are related by partial identity or similarity at one or more blocks or regions within said molecules or sequences.

The term "hybridization" as used herein refers to a process in which a single-stranded nucleic acid molecule joins with a complementary strand through nucleotide base pairing. "Selective hybridization" refers to hybridization under conditions of high stringency. The degree of hybridization depends upon, for example, the degree of homology, the stringency of hybridization, and the length of hybridizing strands.

"Isolated nucleic acid compound" refers to any RNA or DNA sequence, however constructed or synthesized, which is locationally distinct from its natural location.

A "nucleic acid probe" or "probe" as used herein is a labeled nucleic acid compound which hybridizes with another nucleic acid compound. "Nucleic acid probe" means a single stranded nucleic acid sequence that will combine with a complementary or partially complementary single stranded target nucleic acid sequence to form a double-stranded molecule. A nucleic acid probe may be an oligonucleotide or a nucleotide polymer. A probe will usually contain a detectable moiety which may be attached to the end(s) of the probe or be internal to the sequence of the probe.

The term "orthologue" or "orthologous" refers to two or more genes or proteins from different organisms that exhibit sequence homology.

The term "paralogue" or "paralogous" refers to two or more genes or proteins within a single organism that exhibit sequence homology.

The term "plasmid" refers to an extrachromosomal genetic element. The plasmids disclosed herein are commercially available, publicly available on an unrestricted basis, or can be constructed from readily available plasmids in accordance with published procedures.

A "primer" is a nucleic acid fragment which functions as an initiating substrate for enzymatic or synthetic elongation of, for example, a nucleic acid molecule.

The term "promoter" refers to a nucleic acid sequence that directs transcription, for example, of DNA to RNA. An inducible promoter is one that is regulatable by environmental signals, such as carbon source, heat, or metal

ions, for example. A constitutive promoter generally operates at a constant level and is not regulatable.

"Recombinant DNA cloning vector" as used herein refers to any autonomously replicating agent, including, but not limited to, plasmids and phages, comprising a DNA molecule to which one or more additional DNA segments can or have been incorporated.

The term "recombinant DNA expression vector" or "expression vector" as used herein refers to any recombinant DNA cloning vector, for example a plasmid or phage, in which a promoter and other regulatory elements are present thereby enabling transcription of an inserted DNA, which may encode a protein.

The term "stringency" refers to hybridization conditions. High stringency conditions disfavor non-homologous base pairing. Low stringency conditions have the opposite effect. Stringency may be altered, for example, by temperature and salt concentration.

"Low stringency" conditions comprise, for example, a temperature of about 37° C or less, a formamide concentration of less than about 50%, and a moderate to low salt (SSC) concentration; or, alternatively, a temperature of about 50° C or less, and a moderate to high salt (SSPE) concentration, for example 1M NaCl.

"High stringency" conditions comprise, for example, a temperature of about 42° C or less, a formamide concentration of less than about 20%, and a low salt (SSC) concentration; or, alternatively, a temperature of about 65° C, or less, and a low salt (SSPE) concentration. For example, high stringency conditions comprise hybridization

in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (Ausubel, F.M. *et al.* Current Protocols in Molecular Biology, Vol. I, 1989; Green Inc. New York, at 2.10.3).

The symbol "N" in a nucleic acid sequence refers to adenine ("A"), guanine ("G"), cytosine ("C"), thymine (T"), or uracil ("U").

"SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0.

"SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 9mM Na_2HPO_4, 0.9 mM NaH_2PO_4 and 1 mM EDTA, pH 7.4.

"Substantially pure," used in reference to a peptide or protein, means separation from other cellular and non-cellular molecules, including other protein molecules. A substantially pure preparation would be about at least 85% pure; preferably about at least 95% pure. A "substantially pure" protein can be prepared by a variety of techniques, well known to the skilled artisan, including, for example, the IMAC protein purification method.

"Treating" as used herein describes the management and care of a patient for the purpose of combating the disease, condition, or disorder and includes the administration of a protein of the present invention to prevent the onset of the symptoms or complications, alleviating the symptoms or complications, or eliminating the disease, condition, or disorder. Treating as used herein includes the administration of the protein for cosmetic

purposes. A cosmetic purpose seeks to control, for example, the weight of a mammal to improve bodily appearance.

The term "vector" as used herein refers to a nucleic acid compound used for introducing exogenous or endogenous DNA into host cells. A vector comprises a nucleotide sequence which may encode one or more protein molecules. Plasmids, cosmids, viruses, and bacteriophages, in the natural state or which have undergone recombinant engineering, are examples of commonly used vectors.

The various restriction enzymes disclosed and described herein are commercially available and the manner of use of said enzymes including reaction conditions, cofactors, and other requirements for activity are well known to one of ordinary skill in the art. Reaction conditions for particular enzymes were carried out according to the manufacturer's recommendation.

The TRAILLK-2 gene encodes a novel, membrane-bound protein that is related to the TNF family. The TRAILLK-2 cDNA comprises a DNA sequence specified herein by SEQ ID NO:1. The coding region begins at base pair 49 of SEQ ID NO:1 and extends through base pair 798 of SEQ ID NO:1. Those skilled in the art will recognize that owing to the degeneracy of the genetic code, numerous "silent" substitutions of nucleotide base pairs could be introduced into the sequence identified as SEQ ID NO:1 without altering the identity of the encoded amino acid(s) or protein product. All such substitutions are intended to be within the scope of the invention.

Also contemplated by the present invention are related proteins and related functional fragments such as, for

example, smaller alternatively spliced forms. Related proteins comprise a genus in which amino acid substitutions of the primary sequence disclosed in SEQ ID NO:2 is altered by substitution, or replacement, or deletion, or insertion at one or more amino acid positions, such that TNF ligand function is maintained.

Functional fragments are conveniently identified as fragments of an intact TRAILLK-2 protein that retain the capacity to induce apoptosis.

Amino acid substitution modifications can be made in accordance with the following Table.

ORIGINAL RESIDUE	EXEMPLARY SUBSTITUTIONS
ALA	SER
ARG	LYS
ASN	GLN; HIS
ASP	GLU
CYS	SER
GLN	ASN
GLU	ASP
GLY	PRO
HIS	ASN, GLN
ILE	LEU, VAL
LEU	ILE, VAL
LYS	ARG, GLN, GLU
MET	LEU, ILE
PHE	MET, LEU, TYR
SER	THR
THR	SER
TRP	TYR
TYR	TRP, PHE
VAL	ILE, LEU

Fragments of proteins

One embodiment of the instant invention provides fragments of the proteins disclosed that may or may not be biologically active. Such fragments are useful, for example, as an antigen for producing an antibody to said proteins.

Fragments of the proteins disclosed herein may be generated by any number of suitable techniques, including

chemical synthesis of any portion of SEQ ID NO:2, proteolytic digestion of SEQ ID NO:2, or most preferably, by recombinant DNA mutagenesis techniques, well known to the skilled artisan. *See. e.g.* K. Struhl, "Reverse biochemistry: Methods and applications for synthesizing yeast proteins *in vitro*," *Meth. Enzymol.* 194, 520-535. For example, in a preferred method, a nested set of deletion mutations are introduced into the intact gene (SEQ ID NO:1) encoding TRAILLK-2 such that varying amounts of the protein coding region are deleted, either from the amino terminal end, or from the carboxyl end of the protein molecule. This method can also be used to create internal fragments of the intact protein in which both the carboxyl and amino terminal ends are removed. Several appropriate nucleases can be used to create such deletions, for example *Bal*31, or in the case of a single stranded nucleic acid molecule, mung bean nuclease. For simplicity, it is preferred that the TRAILLK-2 gene be cloned into a single-stranded cloning vector, such as bacteriophage M13, or equivalent. If desired, the resulting gene deletion fragments can be subcloned into any suitable vector for propagation and expression of said fragments in any suitable host cell.

The present invention also provides fragments of the proteins disclosed herein wherein said fragments retain receptor activity. As used herein, "functional fragments" refer to fragments of SEQ ID NO:2 that retain and exhibit, under appropriate conditions, measurable biological activity, for example, the capacity to induce apoptosis.

Functional fragments of the proteins disclosed herein may be produced as described above, preferably using

cloning techniques to engineer smaller versions of the intact gene, lacking sequence from the 5' end, the 3' end, from both ends, or from an internal site. Fragments may be tested for biological activity using any suitable assay, for example, the ability of a protein fragment to induce apoptosis, *in vivo* or *in vitro*.

Gene Isolation Procedures

Those skilled in the art will recognize that the TRAILLK-2 gene could be obtained by a plurality of recombinant DNA techniques including, for example, hybridization, polymerase chain reaction (PCR) amplification, or de novo DNA synthesis. (*See e.g.*, T. Maniatis *et al.* Molecular Cloning: A Laboratory Manual, 2d Ed. Chap. 14 (1989)).

Methods for constructing cDNA libraries in a suitable vector such as a plasmid or phage for propagation in procaryotic or eucaryotic cells are well known to those skilled in the art. [*See e.g.* Maniatis *et al. Supra*]. Suitable cloning vectors are well known and are widely available.

The TRAILLK-2 gene or fragment thereof can be isolated a tissue in which said gene is expressed, for example, breast tissue. In one method, mRNA is isolated from a suitable tissue, and first strand cDNA synthesis is carried out. A second round of DNA synthesis can be carried out for the production of the second strand. If desired, the double-stranded cDNA can be cloned into any suitable vector, for example, a plasmid, thereby forming a cDNA library. Oligonucleotide primers targeted to any suitable region of

SEQ ID NO:1 can be used for PCR amplification of TRAILLK-2. *See e.g.* PCR Protocols: A Guide to Method and Application, Ed. M. Innis et al., Academic Press (1990). The PCR amplification comprises template DNA, suitable enzymes, primers, and buffers, and is conveniently carried out in a DNA Thermal Cycler (Perkin Elmer Cetus, Norwalk, CT). A positive result is determined by detecting an appropriately-sized DNA fragment following agarose gel electrophoresis.

Protein Production Methods

One embodiment of the present invention relates to the substantially purified protein encoded by the TRAILLK-2 gene.

Skilled artisans will recognize that the proteins of the present invention can be synthesized by a number of different methods, such as chemical methods well known in the art, including solid phase peptide synthesis or recombinant methods. Both methods are described in U.S. Patent 4,617,149, incorporated herein by reference.

The principles of solid phase chemical synthesis of polypeptides are well known in the art and may be found in general texts in the area. *See, e.g.*, H. Dugas and C. Penney, Bioorganic Chemistry (1981) Springer-Verlag, New York, 54-92. For example, peptides may be synthesized by solid-phase methodology utilizing an Applied Biosystems 430A peptide synthesizer (Applied Biosystems, Foster City, CA) and synthesis cycles supplied by Applied Biosystems.

The proteins of the present invention can also be produced by recombinant DNA methods using the cloned TRAILLK-2 gene. Recombinant methods are preferred if a high

yield is desired. Expression of the cloned gene can be carried out in a variety of suitable host cells, well known to those skilled in the art. For this purpose, the TRAILLK-2 gene is introduced into a host cell by any suitable means, well known to those skilled in the art. While chromosomal integration of the cloned gene is within the scope of the present invention, it is preferred that the gene be cloned into a suitable extra-chromosomally maintained expression vector so that the coding region of the TRAILLK-2 gene is operably-linked to a constitutive or inducible promoter.

The basic steps in the recombinant production of the TRAILLK-2 protein are:

a) constructing a natural, synthetic or semi-synthetic DNA encoding TRAILLK-2 protein;

b) integrating said DNA into an expression vector in a manner suitable for expressing the TRAILLK-2 protein, either alone or as a fusion protein;

c) transforming or otherwise introducing said vector into an appropriate eucaryotic or prokaryotic host cell forming a recombinant host cell,

d) culturing said recombinant host cell in a manner to express the TRAILLK-2 protein; and

e) recovering and substantially purifying the TRAILLK-2 protein by any suitable means, well known to those skilled in the art.

Expressing Recombinant TRAILLK-2 Protein in Procaryotic and Eucaryotic Host Cells

Procaryotes may be employed in the production of recombinant TRAILLK-2 protein. For example, the *Escherichia coli* K12 strain 294 (ATCC No. 31446) is particularly useful for the prokaryotic expression of foreign proteins. Other strains of *E. coli*, bacilli such as *Bacillus subtilis*, enterobacteriaceae such as *Salmonella typhimurium* or *Serratia marcescans*, various Pseudomonas species and other bacteria, such as *Streptomyces*, may also be employed as host cells in the cloning and expression of the recombinant proteins of this invention.

Promoter sequences suitable for driving the expression of genes in procaryotes include b -lactamase [*e.g.* vector pGX2907, ATCC 39344, contains a replicon and b -lactamase gene], lactose systems [Chang et al., *Nature* (London), 275:615 (1978); Goeddel et al., *Nature* (London), 281:544 (1979)], alkaline phosphatase, and the tryptophan (trp) promoter system [vector pATH1 (ATCC 37695)], which is designed to facilitate expression of an open reading frame as a trpE fusion protein under the control of the trp promoter. Hybrid promoters such as the tac promoter (isolatable from plasmid pDR540, ATCC-37282) are also suitable. Still other bacterial promoters, whose nucleotide sequences are generally known, may be ligated to DNA encoding the protein of the instant invention, using linkers

or adapters to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno sequence operably-linked to the DNA encoding the desired polypeptides. These examples are illustrative rather than limiting.

The proteins of this invention may be synthesized either by direct expression or as a fusion protein comprising the protein of interest as a translational fusion with another protein or peptide which may be removable by enzymatic or chemical cleavage. It is often observed in the production of certain peptides in recombinant systems that expression as a fusion protein prolongs the life span, increases the yield of the desired peptide, or provides a convenient means of purifying the protein. This is particularly relevant when expressing mammalian proteins in procaryotic hosts. A variety of peptidases (e.g. enterokinase and thrombin) which cleave a polypeptide at specific sites or digest the peptides from the amino or carboxy termini (*e.g.* diaminopeptidase) of the peptide chain are known. Furthermore, particular chemicals (*e.g.* cyanogen bromide) will cleave a polypeptide chain at specific sites. The skilled artisan will appreciate the modifications necessary to the amino acid sequence (and synthetic or semi-synthetic coding sequence if recombinant means are employed) to incorporate site-specific internal cleavage sites. *See e.g.*, P. Carter, "Site Specific Proteolysis of Fusion Proteins", Chapter 13, in Protein Purification: From Molecular Mechanisms to Large Scale Processes, American Chemical Society, Washington, D.C. (1990).

In addition to procaryotes, a variety of amphibian expression systems such as frog oocytes, and mammalian cell systems can be used. The choice of a particular host cell depends to some extent on the particular expression vector used. Exemplary mammalian host cells suitable for use in the present invention include HepG-2 (ATCC HB 8065), CV-1 (ATCC CCL 70), LC-MK_2 (ATCC CCL 7.1), 3T3 (ATCC CCL 92), CHO-K1 (ATCC CCL 61), HeLa (ATCC CCL 2), RPMI8226 (ATCC CCL 155), H4IIEC3 (ATCC CCL 1600), C127I (ATCC CCL 1616), HS-Sultan (ATCC CCL 1484), and BHK-21 (ATCC CCL 10), for example.

A wide variety of vectors are suitable for transforming mammalian host cells. For example, the pSV2-type vectors comprise segments of the simian virus 40 (SV40) genome required for transcription and polyadenylation. A large number of plasmid pSV2-type vectors have been constructed, such as pSV2-gpt, pSV2-neo, pSV2-dhfr, pSV2-hyg, and pSV2-b-globin, in which the SV40 promoter drives transcription of an inserted gene. These vectors are widely available from sources such as the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, 20852, or the Northern Regional Research Laboratory (NRRL), 1815 N. University Street, Peoria, Illinois, 61604.

Promoters suitable for expression in mammalian cells include the SV40 late promoter, promoters from eukaryotic genes, such as, for example, the estrogen-inducible chicken ovalbumin gene, the interferon genes, the glucocorticoid-inducible tyrosine aminotransferase gene, the

thymidine kinase gene promoter, and the promoters of the major early and late adenovirus genes.

Plasmid pRSVcat (ATCC 37152) comprises portions of a long terminal repeat of the Rous Sarcoma virus, a virus known to infect chickens and other host cells. This long terminal repeat contains a promoter which is suitable for use in the vectors of this invention. H. Gorman *et al.*, *Proc. Nat. Acad. Sci. (USA)*, 79, 6777 (1982). The plasmid pMSVi (NRRL B-15929) comprises the long terminal repeats of the Murine Sarcoma virus, a virus known to infect mouse and other host cells. The mouse metallothionein promoter has also been well characterized for use in eukaryotic host cells and is suitable for use in the present invention. This promoter is present in the plasmid pdBPV-MMTneo (ATCC 37224) which can serve as the starting material for the construction of other plasmids of the present invention.

Transfection of mammalian cells with vectors can be performed by a plurality of well known processes including, but not limited to, protoplast fusion, calcium phosphate co-precipitation, electroporation and the like. *See, e.g.*, Maniatis *et al.*, *supra*.

Some viruses also make appropriate vectors. Examples include the adenoviruses; the adeno-associated viruses, the vaccinia virus, the herpes viruses, the baculoviruses, and the rous sarcoma virus, as described in U.S. Patent 4,775,624, incorporated herein by reference.

Eucaryotic microorganisms such as yeast and other fungi are also suitable host cells. The yeast *Saccharomyces cerevisiae* is the preferred eucaryotic microorganism. Other yeasts such as *Kluyveromyces lactis* and *Pichia pastoris* are

also suitable. For expression in *Saccharomyces*, the plasmid YRp7 (ATCC-40053), for example, may be used. See, e.g., L. Stinchcomb *et al.*, *Nature*, 282, 39 (1979); J. Kingsman *et al.*, *Gene*, 7, 141 (1979); S. Tschemper *et al.*, *Gene*, 10, 157 (1980). Plasmid YRp7 contains the TRP1 gene which provides a selectable marker for use in a trp1 auxotrophic mutant.

Purification of Recombinantly-Produced TRAILLK-2 Protein

An expression vector carrying the cloned TRAILLK-2 gene is transformed or transfected into a suitable host cell using standard methods. Cells that contain the vector are propagated under conditions suitable for expression of the recombinant TRAILLK-2 protein. For Example, if the recombinant gene has been placed under the control of an inducible promoter, suitable growth conditions would incorporate the appropriate inducer. The recombinantly-produced protein may be purified from cellular extracts of transformed cells by any suitable means.

In a preferred process for protein purification, the TRAILLK-2 gene is modified at the 5' end to incorporate several histidine residues at the amino terminus of the TRAILLK-2 protein. This "histidine tag" enables a single-step protein purification method referred to as "immobilized metal ion affinity chromatography" (IMAC), essentially as described in U.S. Patent 4,569,794, which hereby is incorporated by reference. The IMAC method enables rapid isolation of substantially pure recombinant TRAILLK-2 protein starting from a crude extract of cells that express a modified recombinant protein, as described above.

Production of Antibodies

The proteins of this invention and fragments thereof may be used in the production of antibodies. The term "antibody" as used herein describes antibodies, fragments of antibodies (such as, but not limited, to Fab, Fab', Fab_2', and Fv fragments), and chimeric, humanized, veneered, resurfaced, or CDR-grafted antibodies capable of binding antigens of a similar nature as the parent antibody molecule from which they are derived. The instant invention also encompasses single chain polypeptide binding molecules.

The production of antibodies, both monoclonal and polyclonal, in animals, especially mice, is well known in the art. *See, e.g.*, C. Milstein, Handbook of Experimental Immunology, (Blackwell Scientific Pub., 1986); J. Goding, Monoclonal Antibodies: Principles and Practice, (Academic Press, 1983). For the production of monoclonal antibodies the basic process begins with injecting a mouse, or other suitable animal, with an immunogen. The mouse is subsequently sacrificed and cells taken from its spleen are fused with myeloma cells, resulting in a hybridoma that reproduces *in vitro*. The population of hybridomas is screened to isolate individual clones, each of which secretes a single antibody species, specific for the immunogen. Each antibody obtained in this way is the clonal product of a single B cell.

Chimeric antibodies are described in U.S. Patent No. 4,816,567, the entire contents of which is herein incorporated by reference. This reference discloses methods and vectors for the preparation of chimeric antibodies. An alternative approach is provided in U.S. Patent No. 4,816,397, the entire contents of which is herein

incorporated by reference. This patent teaches co-expression of the heavy and light chains of an antibody in the same host cell.

The approach of U.S. Patent 4,816,397 has been further refined in European Patent Publication No. 0 239 400. The teachings of this European patent publication are a preferred format for genetic engineering of monoclonal antibodies. In this technology the complementarity determining regions (CDRs) of a human antibody are replaced with the CDRs of a murine monoclonal antibody, thereby converting the specificity of the human antibody to the specificity of a murine antibody.

Single chain antibodies and libraries thereof are yet another variety of genetically engineered antibody technology that is well known in the art. (*See, e.g.* R.E. Bird, *et al.*, *Science* 242:423-426 (1988); PCT Publication Nos. WO 88/01649, WO 90/14430, and WO 91/10737. Single chain antibody technology involves covalently joining the binding regions of heavy and light chains to generate a single polypeptide chain. The binding specificity of the intact antibody molecule is thereby reproduced on a single polypeptide chain.

The antibodies contemplated by this invention are useful in diagnostics, therapeutics or in diagnostic/therapeutic combinations.

The proteins of this invention or suitable fragments thereof can be used to generate polyclonal or monoclonal antibodies, and various inter-species hybrids, or humanized antibodies, or antibody fragments, or single-chain antibodies. The techniques for producing antibodies are well

known to skilled artisans. (*See e.g.* A.M. Campbell, Monoclonal Antibody Technology: Laboratory Techniques in Biochemsitry and Molecular Biology, Elsevier Science Publishers, Amsterdam (1984); Kohler and Milstein, *Nature* 256, 495-497 (1975); Monoclonal Antibodies: Principles & Applications Ed. J.R.Birch & E.S. Lennox, Wiley-Liss, 1995.

A protein used as an immunogen may be modified or administered in an adjuvant, by subcutaneous or intraperitoneal injection into, for example, a mouse or a rabbit. For the production of monoclonal antibodies, spleen cells from immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag14 cells, and allowed to become monoclonal antibody producing hybridoma cells in the manner known to the skilled artisan. Hybridomas that secrete a desired antibody molecule can be screened by a variety of well known methods, for example ELISA assay, western blot analysis, or radioimmunoassay (Lutz *et al. Exp. Cell Res.* 175, 109-124 (1988); Monoclonal Antibodies: Principles & Applications Ed. J.R.Birch & E.S. Lennox, Wiley-Liss, 1995).

For some applications labeled antibodies are desirable. Procedures for labeling antibody molecules are widely known, including for example, the use of radioisotopes, affinity labels, such as biotin or avidin, enzymatic labels, for example horseradish peroxidase, and fluorescent labels, such as FITC or rhodamine (*See e.g.* Enzyme-Mediated Immunoassay, Ed. T. Ngo, H. Lenhoff, Plenum Press 1985; Principles of Immunology and Immunodiagnostics, R.M. Aloisi, Lea & Febiger, 1988).

Labeled antibodies are useful for a variety of diagnostic applications. In one embodiment the present

invention relates to the use of labeled antibodies to detect the presence of TRAILLK-2. Alternatively, the antibodies could be used in a screen to identify potential modulators of TRAILLK-2. For example, in a competitive displacement assay, the antibody or compound to be tested is labeled by any suitable method. Competitive displacement of an antibody from an antibody-antigen complex by a test compound such that a test compound-antigen complex is formed provides a method for identifying compounds that bind TRAILLK-2.

Other embodiments of the present invention comprise isolated nucleic acid sequences that encode SEQ ID NO:2, or related nucleic acids that are at least about 75% identical to SEQ ID NO:1, or to their complementary sequence, or nucleic acids that hybridize to SEQ ID NO:1 under low or high stringency conditions. Such sequences may come, for example, from paralogous or orthologous genes.

The TRAILLK-2 gene (viz. SEQ ID NO:1) and related nucleic acid molecules that encode SEQ ID NO:2, or functional fragments thereof, may be produced by chemical synthetic methods. The synthesis of nucleic acids is well known in the art. *See, e.g.*, E.L. Brown, R. Belagaje, M.J. Ryan, and H.G. Khorana, *Methods in Enzymology*, 68:109-151 (1979). Fragments of the DNA sequence corresponding to the TRAILLK-2 gene could be generated using a conventional DNA synthesizing apparatus, such as the Applied Biosystems Model 380A or 380B DNA synthesizers (Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, CA 94404) using phosphoramidite chemistry, thereafter ligating the fragments so as to reconstitute the entire gene. Alternatively, phosphotriester chemistry may be employed to synthesize the

nucleic acids of this invention. (*See, e.g.*, M.J. Gait, ed., Oligonucleotide Synthesis, A Practical Approach, (1984)).

In an alternative methodology, namely PCR, the DNA sequences disclosed and described herein, comprising, for example, a portion or all of SEQ ID NO:1 can be produced from a plurality of starting materials. For example, starting with a cDNA preparation (e.g. cDNA library) derived from a tissue that expresses the TRAILLK-2 gene, suitable oligonucleotide primers complementary to SEQ ID NO:1 or to any sub-region therein, are prepared as described in U.S. Patent No. 4,889,818, hereby incorporated by reference. Other suitable protocols for the PCR are disclosed in PCR Protocols: A Guide to Method and Applications, Ed. Michael A. Innis et al., Academic Press, Inc. (1990). Using PCR, any region of the TRAILLK-2 gene can be targeted for amplification such that full or partial length gene sequences may be produced.

The ribonucleic acids of the present invention may be prepared using polynucleotide synthetic methods discussed *supra*, or they may be prepared enzymatically, for example, using RNA polymerase to transcribe a TRAILLK-2 DNA template.

The most preferred systems for preparing the ribonucleic acids of the present invention employ the RNA polymerase from the bacteriophage T7 or the bacteriophage SP6. These RNA polymerases are highly specific, requiring the insertion of bacteriophage-specific sequences at the 5' end of the template to be transcribed. *See*, Maniatis *et al.*, *supra*.

This invention also provides nucleic acids, RNA or DNA, that are complementary to SEQ ID NO:1 or SEQ ID NO:4, or fragment thereof.

Nucleic Acid Probes

The present invention also provides probes and primers useful for a variety of molecular biology techniques including, for example, hybridization screens of genomic, subgenomic, or cDNA libraries, as well as hybridization against nucleic acids derived from cell lines or tissues that originate from drug-resistant tumors. Such hybridization screens are useful as methods to identify homologous and/or functionally related sequences from the same or other organisms. A nucleic acid compound comprising SEQ ID NO:1, or a complementary sequence thereof, or a fragment thereof, which is at least 14 base pairs in length, and which will selectively hybridize to human DNA or mRNA encoding TRAILLK-2 protein or fragment thereof, or a functionally related protein, is provided. Preferably, the 14 or more base pair compound is DNA. *See e.g.* B. Wallace and G. Miyada, "Oligonucleotide Probes for the Screening of Recombinant DNA Libraries," In *Meth. Enzym.*, 152, 432-442, Academic Press (1987).

Probes and primers can be prepared by enzymatic or recombinant methods, well known to those skilled in the art (*See e.g.* Sambrook et al. *supra*). A probe may be a single stranded nucleic acid sequence which is complementary in some particular degree to a nucleic acid sequence sought to be detected ("target sequence"). A probe may be labeled with a detectable moiety such as a radio-isotope, antigen, or chemiluminescent moiety. A description of the use of nucleic

acid hybridization as a procedure for the detection of particular nucleic acid sequences is described in U.S. Patent No. 4,851,330 to Kohne, entitled "Method for Detection, Identification and Quantitation of Non-Viral Organisms."

Having the DNA sequence of the present invention allows preparation of relatively short DNA (or RNA) sequences having the ability to specifically hybridize to gene sequences disclosed herein. In these aspects, nucleic acid probes of an appropriate length are prepared. The ability of such nucleic acid probes to specifically hybridize to a polynucleotide encoding a TRAILLK-2 gene or related sequence lends particular utility in a variety of embodiments. Most importantly, the probes may be used in a variety of assays for detecting the presence of complementary sequences in a given sample.

In certain embodiments, it is advantageous to use oligonucleotide primers. The sequence of such primers is designed using a polynucleotide of the present invention for use in detecting, amplifying or mutating a defined segment of a gene or polynucleotide that encodes a TRAILLK-2 polypeptide using PCR technology.

Preferred nucleic acid sequences employed for hybridization studies, or assays, include probe molecules that are complementary to at least an about 14 to an about 70-nucleotide long stretch of a polynucleotide that encodes a TRAILLK-2 polypeptide, such as the nucleotide base sequences designated as SEQ ID NO:1. A length of at least 14 nucleotides helps to ensure that the fragment is of sufficient length to form a duplex molecule

that is both stable and selective. Molecules having complementary sequences over stretches greater than 14 bases in length are generally preferred, though in order to increase stability and selectivity of the hybrid. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 25 to 40 nucleotides, 55 to 70 nucleotides, or even longer where desired. Such fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as the PCR TM technology of U.S. Pat. No. 4,603,102, herein incorporated by reference, or by excising selected DNA fragments from recombinant plasmids containing appropriate inserts and suitable restriction enzyme sites.

The following guidelines are useful for designing probes with desirable characteristics. The extent and specificity of hybridization reactions are affected by a number of factors that determine the sensitivity and specificity of a particular probe, whether perfectly complementary to its target or not. The affect of various experimental parameters and conditions are well known to those skilled in the art.

First, the stability of the probe:target nucleic acid hybrid should be chosen to be compatible with the assay conditions. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs and by designing a probe with an appropriate Tm (i.e. melting temperature). The melting profile, including the Tm of a hybrid comprising an oligonucleotide and target sequence, may be determined using a Hybridization Protection

Assay. The probe should be chosen so that the length and % GC content result in a Tm about 2°-10° C higher than the temperature at which the final assay will be performed. The base composition of the probe is also a significant factor because G-C base pairs exhibit greater thermal stability as compared to A-T base pairs. Thus, hybridization involving complementary nucleic acids of higher G-C content will be more stable at higher temperatures.

The ionic strength and incubation temperature under which a probe will be used should also be taken into account. It is known that hybridization will increase as the ionic strength of the reaction mixture increases, and that the thermal stability of molecular hybrids will increase with increasing ionic strength. On the other hand, chemical reagents such as formamide, urea, DMSO and alcohols, which disrupt hydrogen bonds, increase the stringency of hybridization. Destabilization of hydrogen bonds by such reagents can greatly reduce the Tm. In general, optimal hybridization for synthetic oligonucleotide probes of about 10-50 bases in length occurs approximately 5° C below the melting temperature for a given duplex. Incubation at temperatures below the optimum may allow mismatched base sequences to hybridize and can therefore result in reduced specificity.

The length of the target nucleic acid sequence and, accordingly, the length of the probe sequence can also be important. In some cases, there may be several sequences from a particular region, varying in location and length, which will yield probes with the desired hybridization characteristics. In other cases, one sequence may be

significantly better than another even though the one sequence differs merely by a single base. Finally, there can be intramolecular and intermolecular hybrids formed within a probe if there is sufficient self-complementarity. Such structures can be avoided through careful probe design. Computer programs are available to search for this type of interaction.

A probe molecule may be used for hybridizing to a sample suspected of possessing a TRAILLK-2 or TRAILLK-2-related nucleotide sequence. The hybridization reaction is carried out under suitable conditions of stringency.

Alternatively, such DNA molecules may be used in a number of techniques including their use as: (1) diagnostic tools to detect polymorphisms in DNA samples from a human or other mammal; (2) means for detecting and isolating homologs of TRAILLK-2 and related polypeptides from a DNA library potentially containing such sequences; (3) primers for hybridizing to related sequences for the purpose of amplifying those sequences; and (4) primers for altering the native TRAILLK-2 DNA sequences; as well as other techniques which rely on the similarity of the DNA sequences to those of the TRAILLK-2 DNA segments herein disclosed.

Once synthesized, oligonucleotide probes may be labeled by any of several well known methods. *See e.g.* Maniatis *et.al.*, Molecular Cloning (2d ed. 1989). Useful labels include radioisotopes, as well as non-radioactive reporting groups. Isotopic labels include H^3, S^{35}, P^{32}, I^{125}, Cobalt, and C^{14}. Most methods of isotopic labeling involve the use of enzymes and include methods such as nick-translation, end-labeling, second strand synthesis, and reverse

transcription. When using radio-labeled probes, hybridization can be detected by autoradiography, scintillation counting, or gamma counting. The detection method selected will depend upon the hybridization conditions and the particular radio isotope used for labeling.

Non-isotopic materials can also be used for labeling, and may be introduced internally into the sequence or at the end of the sequence. Modified nucleotides may be incorporated enzymatically or chemically, and chemical modifications of the probe may be performed during or after synthesis of the probe, for example, by the use of non-nucleotide linker groups. Non-isotopic labels include fluorescent molecules, chemiluminescent molecules, enzymes, cofactors, enzyme substrates, haptens or other ligands. In a preferred embodiment of the invention, the length of an oligonucleotide probe is greater than or equal to about 18 nucleotides and less than or equal to about 50 nucleotides. Labeling of an oligonucleotide of the present invention may be performed enzymatically using [^{32}P]-labeled ATP and the enzyme T4 polynucleotide kinase.

Vectors

Another aspect of the present invention relates to recombinant DNA cloning vectors and expression vectors comprising the nucleic acids of the present invention. The preferred nucleic acid vectors are those which comprise DNA. The most preferred recombinant DNA vectors comprise the isolated DNA sequence, SEQ ID NO:1.

The skilled artisan understands that choosing the most appropriate cloning vector or expression vector depends

upon a number of factors including the availability of restriction enzyme sites, the type of host cell into which the vector is to be transfected or transformed, the purpose of the transfection or transformation (e.g., stable transformation as an extrachromosomal element, or integration into the host chromosome), the presence or absence of readily assayable or selectable markers (e.g., antibiotic resistance and metabolic markers of one type and another), and the number of copies of the gene desired in the host cell.

Vectors suitable to carry the nucleic acids of the present invention comprise RNA viruses, DNA viruses, lytic bacteriophages, lysogenic bacteriophages, stable bacteriophages, plasmids, viroids, and the like. The most preferred vectors are plasmids.

When preparing an expression vector the skilled artisan understands that there are many variables to be considered, for example, whether to use a constitutive or inducible promoter. The practitioner also understands that the amount of nucleic acid or protein to be produced dictates, in part, the selection of the expression system. Regarding promoter sequences, inducible promoters are preferred because they enable high level, regulatable expression of an operably-linked gene. The skilled artisan will recognize a number of suitable promoters that respond to a variety of inducers, for example, carbon source, metal ions, and heat. Other relevant considerations regarding an expression vector include whether to include sequences for directing the localization of a recombinant protein. For example, a sequence encoding a signal peptide preceding the

coding region of a gene is useful for directing the extracellular export of a resulting polypeptide.

The present invention also provides a method for constructing a recombinant host cell capable of expressing proteins comprising SEQ ID NO:2, said method comprising transforming or otherwise introducing into a host cell a recombinant DNA vector that comprises an isolated DNA sequence that encodes SEQ ID NO:2. A suitable host cell is any eucaryotic cell that can accomodate high level expression of an exogenously introduced gene or protein, and that will incorporate said protein into its membrane structure. Vectors for expression are those which comprise SEQ ID NO:1 or fragment thereof. Transformed host cells may be cultured under conditions well known to skilled artisans such that SEQ ID NO:2 is expressed, thereby producing a recombinant TRAILLK-2 protein in the recombinant host cell.

For the purpose of identifying compounds having utility as modifiers of apoptosis, it would be desirable to identify compounds that bind the TRAILLK-2 protein and/or modify its activity. For the purpose of identifying or developing inhibitors or other modifiers that, for example, activate or inhibit the proteins disclosed herein, it would be desirable to identify compounds that bind the TRAILLK-2 protein. A method for determining agents that bind the TRAILLK-2 protein comprises contacting the TRAILLK-2 protein with a test compound and monitoring binding by any suitable means.

The instant invention provides a screening system for discovering compounds that bind the TRAILLK-2 protein, said screening system comprising the steps of:

a) preparing TRAILLK-2 protein;

b) exposing said TRAILLK-2 protein to a test compound;

c) quantifying the binding of said compound to TRAILLK-2 protein by any suitable means.

Utilization of the screening system described above provides a means to determine compounds that may alter the biological function of TRAILLK-2. This screening method may be adapted to large-scale, automated procedures such as a PANDEX® (Baxter-Dade Diagnostics) system, allowing for efficient high-volume screening of potential therapeutic agents.

In such a screening protocol TRAILLK-2 is prepared as described herein, preferably using recombinant DNA technology. A test compound is introduced into a reaction vessel containing the TRAILLK-2 protein or fragment thereof. Binding of TRAILLK-2 by a test compound is determined by any suitable means. For example, in one method radioactively-labeled or chemically-labeled test compound may be used. Binding of the protein by the compound is assessed, for example, by quantifying bound label versus unbound label using any suitable method. Binding of a test compound may also be carried out by a method disclosed in U.S. Patent 5,585,277, which hereby is incorporated by reference. In this method, binding of a test compound to a protein is assessed by monitoring the ratio of folded protein to unfolded protein, for example by monitoring sensitivity of

said protein to a protease, or amenability to binding of said protein by a specific antibody against the folded state of the protein.

The foregoing screening methods are useful for identifying a ligand of a TRAILLK-2 protein, as a lead to a pharmaceutical compound for modulating apoptosis. A ligand that binds TRAILLK-2, or related fragment thereof, is identified, for example, by combining a test ligand with TRAILLK-2 under conditions that cause the protein to exist in a ratio of folded to unfolded states. If the test ligand binds the folded state of the protein, the relative amount of folded protein will be higher than in the case of a test ligand that does not bind the protein. The ratio of protein in the folded versus unfolded state is easily determinable by, for example, susceptibility to digestion by a protease, or binding to a specific antibody, or binding to chaperonin protein, or binding to any suitable surface.

The present invention also provides a pharmaceutical composition comprising as the active agent a polypeptide compound represented by SEQ ID NO:2, or a pharmaceutically acceptable non-toxic salt thereof, and a pharmaceutically acceptable solid or liquid carrier. For example, compounds comprising TRAILLK-2 can be admixed with conventional pharmaceutical carriers and excipients, and used in the form of tablets, capsules, elixirs, suspensions, syrups, wafers, and the like. The compositions comprising TRAILLK-2 will contain from about 0.1% to 90% by weight of the active compound, and more generally from about 10% to 30%. The compositions may contain common carriers and excipients such as corn starch or gelatin, lactose, sucrose,

microcrystalline cellulose, kaolin, mannitol, dicalcium phosphate, sodium chloride, and alginic acid. The compounds can be formulated for oral or parenteral administration.

Skilled artisans will recognize that IC_{50} values are dependent on the selectivity of the compound tested. For example, a compound with an IC_{50} which is less than 10 nM is generally considered an excellent candidate for drug therapy. However, a compound which has a lower affinity, but is selective for a particular target, may be an even better candidate. The skilled artisan will recognize that any information regarding the binding potential, inhibitory activity, or selectivity of a particular compound is useful toward the development of pharmaceutical products.

The following examples more fully describe the present invention. Those skilled in the art will recognize that the particular reagents, equipment, and procedures described are merely illustrative and are not intended to limit the present invention in any manner.

EXAMPLE 1

RT-PCR Amplification of TRAILLK-2 Gene from mRNA

A TRAILLK-2 gene is isolated by reverse transcriptase PCR (RT-PCR) using conventional methods. Total RNA from a tissue that expresses the TRAILLK-2 gene, is prepared using standard methods. First strand cDNA synthesis is achieved using a commercially available kit (SuperScript™ System; Life Technologies) by PCR in conjunction with specific primers directed at any suitable region of SEQ ID NO:1, for example, at the ATG start site at base pair 49, and at the TGA stop site after base pair 798.

Amplification is carried out by adding to the first strand cDNA (dried under vacuum): 8 µl of 10X synthesis buffer (200 mM Tris-HCl, pH 8.4; 500 mM KCl, 25 mM $MgCl_2$, 1 ug/ul BSA); 68 µl distilled water; 1 µl each of a 10 uM solution of each primer; and 1 µl Taq DNA polymerase (2 to 5 U/µl). The reaction is heated at 94° C for 5 min. to denature the RNA/cDNA hybrid. Then, 15 to 30 cycles of PCR amplification are performed using any suitable thermal cycle apparatus. The amplified sample may be analyzed by agarose gel electrophoresis to check for an appropriately-sized fragment.

EXAMPLE 2

Production of a Vector for Expressing TRAILLK-2 in a Host Cell

An expression vector suitable for expressing TRAILLK-2 or fragment thereof in a variety of procaryotic host cells, such as *E. coli* is easily made. The vector contains an origin of replication (Ori), an ampicillin resistance gene (Amp) useful for selecting cells which have incorporated the vector following a tranformation procedure, and further comprises the T7 promoter and T7 terminator sequences in operable linkage to a TRAILLK-2 coding region. Plasmid pET11A (obtained from Novogen, Madison WI) is a suitable parent plasmid. pET11A is linearized by restriction with endonucleases NdeI and BamHI. Linearized pET11A is ligated to a DNA fragment bearing NdeI and BamHI sticky ends and comprising the coding region of the TRAILLK-2 gene as disclosed by SEQ ID NO:1 or fragment thereof.

The TRAILLK-2 gene used in this construction may be slightly modified at the 5' end (amino terminus of encoded protein) in order to simplify purification of the encoded protein product. For this purpose, an oligonucleotide encoding 8 histidine residues is inserted after the ATG start codon. Placement of the histidine residues at the amino terminus of the encoded protein serves to enable the IMAC one-step protein purification procedure.

EXAMPLE 3

Recombinant Expression and Purification of TRAILLK-2 Protein

An expression vector that carries an ORF encoding TRAILLK-2 or fragment thereof and which ORF is operably-linked to an expression promoter is transformed into *E. coli* BL21 (DE3) (*hsdS gal* lcIts857 *ind1Sam7nin5lac*UV5-T7gene 1) using standard methods. Transformants, selected for resistance to ampicillin, are chosen at random and tested for the presence of the vector by agarose gel electrophoresis using quick plasmid preparations. Colonies which contain the vector are grown in L broth and the protein product encoded by the vector-borne ORF is purified by immobilized metal ion affinity chromatography (IMAC), essentially as described in US Patent 4,569,794.

Briefly, the IMAC column is prepared as follows. A metal-free chelating resin (e.g. Sepharose 6B IDA, Pharmacia) is washed in distilled water to remove preservative substances and infused with a suitable metal ion [e.g. Ni(II), Co(II), or Cu(II)] by adding a 50mM metal chloride or metal sulfate aqueous solution until about 75% of the interstitial spaces of the resin are saturated with

colored metal ion. The column is then ready to receive a crude cellular extract containing the recombinant protein product.

After removing unbound proteins and other materials by washing the column with any suitable buffer, pH 7.5, the bound protein is eluted in any suitable buffer at pH 4.3, or preferably with an imidizole-containing buffer at pH 7.5.

EXAMPLE 4

Tissue Distributuion of TRAILLK-2 mRNA

The presence of TRAILLK-2 mRNA in a variety of human tissues was analyzed by Northern analysis. Total RNA from different tissues or cultured cells was isolated by a standard guanidine chloride/phenol extraction method, and poly-A^+ RNA was isolated using oligo(dT)-cellulose type 7 (Pharmacia). Electrophoresis of RNA samples was carried out in formaldehyde followed by capillary transfer to Zeta-Probe TM nylon membranes (Bio-Rad, Hercules, Calif.). SEQ ID NO:1 was the template for generating probes using a MultiPrime™ random priming kit (Amersham, Arlington Heights, Ill.). The efficiency of the labeling reaction was approximately 4 x 10^{10} cpm incorporated per µg of template. The hybridization buffer contained 0.5M sodium phosphate, 7% SDS (wt/vol), 1% BSA (wt/vol), and 1 mM EDTA. Prehybridization was carried out in hybridization buffer at 65° C for 2 h and ^{32}P-labeled probe was added and incubation continued overnight. The filters were washed in Buffer A (40 mM sodium phosphate pH 7.2, 5% SDS [wt/vol], 0.5% BSA [wt/vol], and 1 mM EDTA) at 65° C for 1 h, and then in Buffer B (40 mM sodium phosphate, pH 7.2, 1% SDS [wt/vol], and 1 mM EDTA) at 65° C for 20 min.

The filters were air-dried and exposed to Kodak X-OMAT AR film at -80° C with an intensifying screen.

EXAMPLE 5

Detecting Ligands that Bind TRAILLK-2 Using a Chaperonin Protein Assay

The wells of an ELISA plate are coated with chaperonin by incubation for several hours with a 4 ug/ml solution of the protein in Tris-buffered Saline (TBS: 10 mM Tris-HCl, pH7.5, 0.2M NaCl). The plates are then washed 3 times with TBS containing 0.1% Tween-20 (TBST). Then, a mixture of TRAILLK-2 protein (sufficient amount to saturate about 50% of the binding sites on chaperonin) and test compound (10^{-9} to 10^{-5} M) in about 50 µl volume is added to each well of the plate for an incubation of about 60 minutes. Aliquots of the well solutions are then transferred to the wells of fresh plates and incubated for 60 minutes at room temperature, followed by 3 washes with TBST. Next, about 50 µl of an antibody specific for TRAILLK-2 plus 5% nonfat dry milk are added to each well for a 30 minute incubation at room temperature. After washing, about 50 µl of goat anti-rabbit IgG alkaline phosphatase conjugate at an appropriate dilution in TBST plus 5% nonfat dry milk are added to each will and incubated 30 minutes at room temperature. The plates are washed again with TBST and 0.1 ml of 1 mg/ml p-nitrophenylphosphate in 0.1% diethanolamine is added. Color development (proportional to bound alkaline phosphatase antibody conjugate) is monitored with an ELISA plate reader. When test ligand binding has occurred, ELISA analysis

reveals TRAILLK-2 in solution at higher concentrations than in the absence of test ligand.

EXAMPLE 6

Production of an Antibody to TRAILLK-2 Protein

Substantially pure TRAILLK-2 protein or fragment thereof is isolated from transfected or transformed cells using any of the well known methods in the art, or by a method specifically disclosed herein. Concentration of protein in a final preparation is adjusted, for example, by filtration through an Amicon filter device such that the level is about 1 to 5 ug/ml. Monoclonal or polyclonal antibody can be prepared as follows.

Monoclonal antibody can be prepared from murine hybridomas according to the method of Kohler and Milstein (*Nature*, 256, 495, 1975), or a modified method thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the protein or fragment thereof, or fusion peptide thereof, over a period of a few weeks. The mouse is then sacrificed and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells. Fused cells that produce antibody are identified by any suitable immunoassay, for example, ELISA, as described in E. Engvall, *Meth. Enzymol.*, 70, 419, 1980.

Polyclonal antiserum can be prepared by well known methods (*See e.g.* J. Vaitukaitis *et.al. Clin. Endocirnol. Metab.* 33, 988, 1971) that involve immunizing suitable animals with the proteins, fragments thereof, or fusion proteins thereof, disclosed herein. Small doses (e.g.

nanogram amounts) of antigen administered at multiple intradermal sites appears to be the most reliable method

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CLAIMS

1. A substantially pure protein having the amino acid sequence which is SEQ ID NO: 2.

2. An isolated nucleic acid compound encoding the protein of Claim 1, said protein having the amino acid sequence that is SEQ ID NO: 2.

3. An isolated nucleic acid compound that encodes a protein having TNF family ligand activity wherein said nucleic acid hybridizes to SEQ ID NO:1 under high stringency conditions.

4. A vector comprising an isolated nucleic acid compound of Claim 2 or Claim 3.

5. A vector, as in Claim 4, wherein said isolated nucleic acid compound is operably-linked to a promoter sequence.

6. A host cell containing a vector of Claim 4 or Claim 5.

7. A method for constructing a recombinant host cell having the potential to express SEQ ID NO:2, said method comprising introducing into said host cell by any suitable means a vector of Claim 5.

8. A method for identifying compounds that bind a protein identified herein as SEQ ID NO:2, comprising the steps of:

a) admixing a substantially purified preparation of a protein comprising SEQ ID NO:2 with a test compound; and

b) monitoring by any suitable means a binding interaction between said protein and said compound.

9. A pharmaceutical formulation comprising as an active ingredient TRAILLK-2 protein, associated with one or more pharmaceutically acceptable carriers, excipients, or diluents thereof.

10. TRAILLK-2 or an analog thereof for use in treating cancer in a patient in need thereof.

ABSTRACT

The invention provides isolated nucleic acid compounds, proteins and fragments thereof, said proteins being related to the family of TNF ligands. Also provided are vectors and transformed heterologous host cells for expressing the protein and a method for identifying compounds that bind and/or modulate the activity of said proteins, and a method for treating cancerous growths in a patient in need thereof.